GENETIC ANALYSIS OF PLANT ARCHITECTURE IN SORGHUM

by

WENQIAN KONG

(Under the Direction of Andrew H. Paterson)

ABSTRACT

Plant architecture is the three-dimensional organization of the plant body. Above-ground architecture is determined by the size, shape and positions of leaves, stems and flowers; and underground architecture by roots and rhizomes. We conduct quantitative trait locus (QTL) mapping in a recombinant inbred line (RIL) population derived from two morphologically distinct parents, *S. bicolor* and *S. propinquum*, to identify genomic regions responsible for vegetative branching pattern and rhizomatousness, respectively, and to facilitate comparisons between these two traits and among their respective components. We show overlapping genetic control of above-ground and below-ground plant architecture, validate quantitative trait loci (QTLs) previously reported in an F2 population of the same cross, and discover additional QTLs. Understanding the genetic determinants of plant architecture sheds new light on genetic manipulation of plants for a variety of purposes, and advances progress towards identification of underlying genes that may contribute to plant growth regulation.

INDEX WORDS: Vegetative branching; Rhizomes; Quantitative trait loci

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Sorghum in a nutshell

With centers of diversity and domestication in northeastern Africa, sorghum is one of only a few African crops achieving extensive distribution, and also retains immense morphological variation. Although archaeological documentation for the time of domestication and cultivation is still lacking and controversial, it is believed that early domestication and cultivation of sorghum could have dated back to 3000 B.C., and that this plant was introduced to China and India through the trade routes 2000 years ago (Dewet and Huckabay 1967). Selection for widely adapted African wild sorghums for different uses (construction material, food, forage and beverage) in early stages, followed by formation of new hybrid combinations as a result of human migration, may have contributed to a wide range of variation among sorghums grown across a large area of the African continent.

To date, sorghum ranks as the fifth most important cereal crop based on production quantity. In 2010, the world's sorghum production was 55.7 million tons from approximately 40.93 million ha of land (http://faostat.fao.org). Among many countries that grow sorghum, the United States, India, Nigeria, Argentina and Ethiopia have the largest production. The gross production of sorghum worldwide in 2010 was worth $14.5 billion, following rice ($249.3 billion), corn ($190.7 billion), wheat ($151.8 billion) and barley ($23.1 billion) (http://faostat.fao.org).

Undoubtedly, sorghum plays a vital role in helping to relieve world poverty in adverse conditions. Tolerant to low water inputs, sorghum has the largest gross production value in sub-Sahelian Africa where the water supply is limited and the soil is unsuitable for cultivation of many other crops. Sorghum has been used for a variety of purposes such as food, forage, sugar, and fiber ('broomcorn'). Increasing demand for food with ongoing population growth, and new challenges such as climate change and demand for bioenergy with limited inputs, suggest that sorghum will become of greater interest and importance.

Sorghum is a promising biofuel plant. In 2007, US ethanol production consumed 15-25% of corn grain production (Rooney, Blumenthal et al. 2007). Sorghum, currently the second source of grain-based ethanol in the USA, is an outstanding alternative to corn due to its lower usage for food and adaptability to marginal lands. As a potential successor to grain-based biofuel production, lignocellulosic biomass production is advantageous, especially with greater utilization of agriculturally degraded lands via a sustainable, perennial system. Sweet sorghum, with high stalk sugar content, has been planted as an alternative sugar source and may be of growing interest in biofuel production (Murray, Rooney et al. 2008; Murray, Sharma et al. 2008).

Sorghum bicolor has a relatively small genome size (~730 Mb), and has long been an attractive model for understanding functional genomics, biochemical pathways and evolution in cereal crops (Paterson, Bowers et al. 2009). Sorghum uses 'C4' photosynthesis, which has a higher CO_2 assimilation rate above 30°C and is better adapted to subtropical and tropical areas than 'C3' photosynthesis used by rice. The study of sorghum sheds light upon many other closely related crops such as maize (Zea) and the Saccharinae clade of grasses. Sorghum shared a common ancestor with Zea ~12 mya (Swigonova, Lai et al. 2004), and with Saccharum

(sugarcane) ~7.7 mya (Jannoo, Grivet et al. 2007), much more recently than rice, ~42 mya (Paterson, Bowers et al. 2004).

Sorghum offers a great opportunity to study invasive plants and weeds. *Sorghum halepense* (2n=40), an invasive and noxious species across much of the world, disperses widely through vegetative reproduction by forming abundant and aggressive rhizomes, and also through disarticulation ('shattering') of seeds. It is indigenous to western Asia, and has been introduced or spread to all continents except Antarctica. A naturally formed tetraploid, *S. halepense* is derived from *S. bicolor* and *S. propinquum*, a rhizomatous perennial plant native to Southeast Asia (Paterson, Schertz et al. 1995). Characterizing and understanding the molecular basis and biological pathways of invasiveness in this plant, using rich information from sorghum, may benefit weed control and development of herbicide, since there are few ways to control *S. halepense* in closely related sorghum fields. On the other hand, rhizomes are precious for maintaining thick productive stands of many perennial forage plants such as bermudagrass (*Cynodon dactylon*), growing vigorously to prevent soil erosion, and offering opportunities to improve agricultural productivity.

Genetic mapping in sorghum

Genetic linkage maps are valuable to study the inheritance of a variety of traits, to assist molecular breeding and marker-assisted selection, to conduct map-based cloning and to compare the genetic control of traits between different species. Sorghum genetic mapping was initiated in the early 1990s with the advent of RFLP markers, and numerous genetic maps have been published using different marker systems such as amplified fragment length polymorphism (AFLP), simple sequence repeats (SSRs) and Diversity Array Technology (DArT) markers (Chittenden, Schertz et al. 1994; Pereira, Lee et al. 1994; Xu, Magill et al. 1994; Boivin, Deu et

al. 1999; Bhattramakki, Dong et al. 2000; Kong, Dong et al. 2000; Haussmann, Hess et al. 2002; Menz, Klein et al. 2002; Bowers, Abbey et al. 2003; Wu and Huang 2007; Mace, Xia et al. 2008; Mace, Rami et al. 2009). A high-density genetic map (Bowers, Abbey et al. 2003) provided information aiding in the construction of a physical map (Bowers, Arias et al. 2005) and the assembly of the sorghum reference genome sequences (Paterson, Bowers et al. 2009). However, use of different marker systems and different crosses to produce a number of different genetic linkage maps complicates further applications of the information. Thus, a sorghum consensus map was constructed to bridge different genetic maps, obtain more coverage on sorghum genomes, align QTLs to genomic sequence, and advance further applications such as molecular breeding and positional cloning (Mace, Rami et al. 2009).

QTL mapping in sorghum

Many agronomically important traits, such as yield and quality, are controlled by many genes, and are known as polygenic traits. Quantitative trait locus (QTL) mapping, is a way to identify genomic regions underlying important phenotypic variations. Routinely, researchers assign genetic markers, mostly DNA markers, to chromosomes. The resulting “linkage map” is utilized to estimate the position, number, and mode of gene action of genomic regions that confer quantitative traits.

A number of population structures can be applied to QTL mapping, such as backcross, F2, recombinant inbred line (RIL), and doubled haploid (DH). Parents selected for construction of the population need to differ for alleles conferring one or more traits of interest. The number of individuals in the population generally ranges from 50 to 250 (Mohan, Nair et al. 1997), while high-resolution mapping requires a larger population size. QTL mapping in RILs has many advantages. The RIL population is immortal, and can be replicated in many locations and

different years. Genotype by environment interaction and heritability can be explored with variance components methods.

QTL mapping in sorghum has addressed both traditionally important traits such as yield and plant morphology (Rami, Dufour et al. 1998; Srinivas, Satish et al. 2009), and many other traits that are unique to sorghum related to disease (Agrama, Widle et al. 2002; McIntyre, Hermann et al. 2004; Mcintyre, Casu et al. 2005; Totad, Fakrudin et al. 2005; Singh, Chaudhary et al. 2006; Aljanabi, Parmessur et al. 2007; Parh, Jordan et al. 2008), abiotic stress resistance (Tuinstra, Ejeta et al. 1998; Crasta, Xu et al. 1999; Xu, Subudhi et al. 2000; Kebede, Subudhi et al. 2001; Sanchez, Subudhi et al. 2002), and stay-green (Subudhi, Rosenow et al. 2000; Xu, Subudhi et al. 2000; Haussmann, Mahalakshmi et al. 2002; Kassahun, Bidinger et al. 2010). Demand for bioenergy motivates discovery of QTLs of many sugar related traits, such as biomass composition, sugar content, and brix (Ritter, Jordan et al. 2008; Shiringani, Frisch et al. 2010; Felderhoff, Murray et al. 2012). QTL mapping is especially useful in interspecific populations, and characterization of QTLs controlling traits such as vegetative branching, rhizomatousness, flowering time, shattering and seed size (Lin, Schertz et al. 1995; Paterson, Schertz et al. 1995; Hart, Schertz et al. 2001; Brown, Klein et al. 2006), provides a great opportunity to study the evolution and domestication of sorghum. The Comparative Quantitative Trait Locus Database for Saccharinae Grasses (CSGRqtl) (Zhang, Guo et al. 2013) is an inclusive tool that allows us to align QTLs to their physical positions, facilitate comparisons between Saccharinae crops, and integrate gene annotations, genetic markers and paleo-duplicated regions.

Vegetative branching in grasses

Introduction

Variations in plant architecture are important for human utilization of the Poaceae grass family for different purposes (McSteen 2009) (Figure 1.1). Cultivated cereal crops, such as rice and maize, have experienced selection for quick production of synchronized tillers and flowering heads suitable for mechanical harvesting of seed/grain. On the other hand, forage crops, turf grasses and a number of wild plants are selected for producing abundant tillers and rhizomes for perennial growth and grazing resistance.

Studying the genetic basis of vegetative branching in cereal crops has been a fertile field (Wang and Li 2006; Doust 2007; Wang and Li 2011). Vegetative branching is largely genetically controlled, though the causal biochemical pathways may differentiate the grass family from dicot model plants (Doust 2007). In addition to genetic factors, environmental factors such as density, temperature, humidity and nutrition, play a vital role in regulating branching patterns (Moulia, Loup et al. 1999; Lafarge, Broad et al. 2002; Doust and Kellogg 2006). For example, increasing plant density will lead to fewer branches, perhaps due to shading and competition for nutrition. Hormones are another important factor in regulating vegetative branching (McSteen 2009). Auxin and cytokinin have long been known to influence the size and kinetics of axillary meristems (Beveridge 2006). The identification of the new plant growth hormone, strigolactone, reveals an additional hormone affecting plant architecture (Gomez-Roldan, Fermas et al. 2008; Agusti, Herold et al. 2011).

Processes of tillering and branching formation

Plant architecture results from the combined developmental control of the shoot apical meristem (SAM) and axillary meristems (AM). SAM, a group of cells at the tip of the primary

axis initiated during embryogenesis, controls the development and elaboration of the primary axis; AM is a group of cells formed in the axils of leaves (McSteen and Leyser 2005). Therefore, SAM controls the development of the primary axis, whereas the majority of diversity and variation of branching are influenced by AM.

During vegetative development, AM first initiates and develops into buds. In grasses, their outgrowth is controlled in a spatial-temporal manner. Tillers are developed from the basal nodes of the plants at early developmental stages and grow out in an acropetal manner. Those tillers developed from the AM can form their own adventitious roots that resemble the primary axis developed from SAM, all contributing to bushy architecture and to yield. Secondary branches emanate higher up basipetally on stems, and their development is usually arrested during bud stages under genetic and hormonal control (Leyser 2003; Leyser 2006). Those branches are essential: for example, in maize, the ears are derived from the AMs a few nodes higher than the basal nodes; in other plants, synchronized secondary branches during harvesting can be a component of yield.

While tillers are usually developed in an upright manner, they can also variously grow in a horizontal manner, resulting in stoloniferous or rhizomatous growth habit. The divergent development of tillers and rhizomes produces two physiologically different organs. Above-ground tillers produce inflorescences and seeds, and are subject to senescence, while rhizomes can store and allocate nutrients for perennial growth under poor conditions even at the expense of seeds in temperate latitudes, facilitating overwintering and rapid growth early in the next season. Since all our major crops are annual, only a few studies have investigated genes controlling the growth habit shift from tillers to rhizomes (Paterson, Schertz et al. 1995; Hu, Tao et al. 2003; Westerbergh and Doebley 2004).

Quantitative studies of vegetative branching

Detecting genomic regions conferring plant architecture has been effective in many modern crop species. One example is the discovery of the domestication related gene *teosinte branched (tb1)* in maize (Doebley, Stec et al. 1995). Teosinte and maize are morphologically different in plant architecture: teosinte bears many lateral branches with each ending in an inflorescence or tassel, and each branch may bear their own ears positions at the nodes along the branches in clusters. Modern cultivated maize has evolved with an increase in apical dominance with one or two lateral branches, i.e. the female inflorescence, occurring two or three nodes from the base of the primary stem. The different architecture of teosinte and maize is largely due to the *tb1* genomic region, which was proved by complementation tests. It is now clear that *tb1* causes decreased apical dominance and profuse lateral branching by regulating axillary meristem outgrowth. This gene was cloned and found to encode a putative helix-loop-helix DNA binding protein in the TCP gene family (Doebley, Stec et al. 1997; Lukens and Doebley 2001). Recent studies have shown that cis-regulatory sequences >41 kb at intergenic regions upstream of *tb1* alter its transcription, supporting the hypothesis that non-coding DNA in many large genomes may regulate gene expression and quantitative phenotypes (Clark, Wagler et al. 2006). Elegant work on *tb1* offers methods and information for identifying and comparing other *tb1*-like genes in many other species as well as genetic engineering of this gene to control the degree of apical dominance.

Quantitative studies in many other crops reveal genomic regions controlling plant architecture, often involving small effect QTLs unlike maize, suggesting that different crops may experience different modification of their architecture. Quantitative study in foxtail millet concludes that some QTLs have a general effect in controlling tillering and secondary branching

while other QTLs contribute specifically to one mechanism. Plant architecture of foxtail millet has experienced selection on different sets of genes from that of maize, with the ortholog of *tb1* only exerting a small effect (Doust, Devos et al. 2004).

Tillering of at least two different populations of sorghum has been studied: in an F2 population derived from two morphologically different parents *Sorghum bicolor* and its wild relative, *Sorghum propinquum*, four QTLs on chromosomes 1, 5, 6, 7 were identified and collectively explained 23.7% of the total genetic variance (Paterson, Schertz et al. 1995). Another quantitative study in a recombinant inbred line (RIL) population from two *Sorghum bicolor* parents, i.e. BTx623 and IS3620C, discovered two QTLs on chromosomes 1 and 6 in two environments, the latter overlapping with the QTL found in the former population (Hart, Schertz et al. 2001; Feltus, Hart et al. 2006).

Forward genetics is also an effective way to discover genomic regions controlling plant architecture in rice, though different populations yield different sets of QTLs (Hu, Tao et al. 2003; Li, Zhou et al. 2006; Onishi, Horiuchi et al. 2007; Jin, Huang et al. 2008) in different developmental stages (Yan, Zhu et al. 1998). The recently found *PROG1* (*PROSTRATE GROWTH 1*) gene on rice chromosome 7 affects tiller angle and tiller number. Encoding a C2H2-type zinc-finger motif, this gene functions in both SAM and AMs and effectively controls both axillary meristem initiation and outgrowth (Jin, Huang et al. 2008).

In partial summary, quantitative studies of plant vegetative branching have provided a considerable amount of information and resources for gene identification and cloning and comparative mapping, exemplified by the maize *tb1* mutant. Because of the labor- intensive nature of quantitative study of this trait, evaluation of vegetative branching is rarely regarded as a

priority. More studies on this trait are needed for comparative mapping and elucidating variation during domestication and crop improvement processes.

Genes controlling axillary meristem initiation

Although voluminous literature has described quantitative studies in plant architecture, few has been focused on identifying single genes. On the other hand, reverse genetics has successfully characterized mutants in rice, maize and barley. Those mutants can be further categorized into genes controlling axillary meristem initiation and genes controlling axillary meristem outgrowth based on their different developmental mechanism. Tillers and branches both arise from axillary buds from leaf axils on the main stem, but their outgrowth and dormancy are also genetically controlled. The functions, biochemical pathways of these mutants and their regulating hormones have been gradually elucidated (Leyser 2003; McSteen 2009; Wang and Li 2011).

One breakthrough is the discovery of the rice *MONOCULM* (*MOC1*) gene, the mutant of which is defective in bearing tillers and branches at both vegetative and reproductive stages (Li, Qian et al. 2003). *MOC1* encodes a GRAS family transcription factor, similar to *LS* in tomatoes and *LAS* in Arabidopsis that controls AM initiation (Groot, Keizer et al. 1994; Greb, Clarenz et al. 2003). The *BA1* gene found in maize is required for both vegetative axillary meristem initiation and for early inflorescence development, the homozygous mutant of which is unable to produce vegetative branches, female ears and a normal male inflorescence (Ritter, Padilla et al. 2002; Gallavotti, Zhao et al. 2004). The *ba1* locus encodes a bHLH domain, which is conserved compared to the *LAX PANICLE* (*LAX*) gene in rice (Komatsu, Maekawa et al. 2003), and *REGULATOR OF AXILLARY MERISTEM FORMATION* (*ROX*) in Arabidopsis (Yang, Wang et al. 2012), while *LAX* affects axillary meristems only in the inflorescence and *ROX* only in

vegetative branches. It is reported that *BIF2* interacts with *BA1* based on yeast two-hybrid screening (Gallavotti, Zhao et al. 2004; Skirpan, Wu et al. 2008), and the double mutant of *ba1* and *bif2* will also affect the axillary meristem initiation during both tillering and inflorescence development. Similar to that of maize, double mutants *lax* and *spa* in rice will lead to defects in both vegetative branching and inflorescence, suggesting the existence of a general regulatory mechanism that controls axillary meristem initiation among the grass family.

Other genes regulating axillary meristem initiation during vegetative development are *REV/OSHB3* that encodes a HD ZIP class III transcription factor (Otsuga, DeGuzman et al. 2001; Itoh, Hibara et al. 2008), and *CUC1, 2, 3/OsTIL1* that encodes a NAC domain transcription factors (Takada, Hibara et al. 2001; Vroemen, Mordhorst et al. 2003; Mao, Ding et al. 2007) in *Arabidopsis* and rice, though over-expression of *OsTIL1* influences the axillary meristem outgrowth (Table 1.1).

Genes controlling axillary meristem outgrowth

Usually, a plant produces more axillary meristems than it uses. Once an axillary meristem has formed, both genetic and environmental factors will determine its dormancy or outgrowth. Stimulation such as mowing or grazing may induce the outgrowth of previously-dormant meristems.. Axillary meristem outgrowth is mostly under hormonal control, especially the trade-off between auxin and cytokinin (Leyser 2003; McSteen 2009). One canonical example of the effect of auxin is that decapitation will lead to growth of additional axillary branches. Mutants of genes controlling auxin and related products have been reported to cause a bushy architecture (Lincoln, Britton et al. 1990; Xu, Zhu et al. 2005; Cheng, Dai et al. 2006). Cytokinin was shown to promote bud outgrowth, but whether it works independently to auxin (Chatfield, Stirnberg et al. 2000), or interacts with auxin (Nordstrom, Tarkowski et al. 2004) is still debated. The

recently found hormone strigolactone (Gomez-Roldan, Fermas et al. 2008; Umehara, Hanada et al. 2008) acts as a new hormone class in regulating above-ground shoot branching. However, the exact hormone levels and their interactions are still under investigation.

A number of studies are focused on genes controlling axillary meristem outgrowth in *Arabidopsis* and rice (Table 1.2) (Wang and Li 2011). The *Arabidopsis more axillary growth* (*max*) pathway (Stirnberg, van de Sande et al. 2002; Sorefan, Booker et al. 2003) is proved to affect the outgrowth of axillary meristems, since the number of axillary meristems of *max* mutants is not suppressed. Tiller *dwarf (d)* mutants of rice have similar phenotypes as *max* mutants, displaying increasing numbers of branches and reducing height. It is suggested that those mutants are deficient in synthesizing strigolactone and their signaling molecules (Gomez-Roldan, Fermas et al. 2008; Umehara, Hanada et al. 2008).

In maize, the best characterized gene is *TB1* that was involved in the domestication from its wild relative teosinte (Hubbard, McSteen et al. 2002). Maize has only a single axis while teosinte is highly branched. *TB1* encodes a TCP transcription factor family member of which an increasing level will suppress outgrowth of buds. The orthologs of maize *TB1*, *OsTB1* in rice and *BRC1* in *Arabidopsis* function similarly to *TB1*, promoting growth arrest of axillary buds (Takeda, Suwa et al. 2003; Aguilar-Martinez, Poza-Carrion et al. 2007). *BRC1* acts downstream of the *MAX* pathway, as double mutants show the *max* phenotype. In addition, *BRC1* is also required for the auxin-mediated pathway. Obviously, *TB1*/*OsTB1*/*BRC1* are involved in a conserved pathway in monocots and dicots, though the growth habit of maize, rice and *Arabidopsis* vary. Therefore, this similar set of genes may reflect the common evolutionary origin of the genes and regulatory elements. Further comparison of orthologous genes in other species may clarify this hypothesis and identify their different growth habits.

It is commonly known that vegetative branching is largely affected by environmental factors, such as density and nutrition (Doust and Kellogg 2006; Kebrom, Burson et al. 2006; Whipple, Kebrom et al. 2011). Maize grassy tiller (*gt1*) responds to shade by enhancing the expression of *gt*, leading to decreased branching. Further, *GT1* acts downstream of *TB1*, and both genes suppress axillary bud outgrowth in response to light capture (Whipple, Kebrom et al. 2011). Similar findings are suggested in sorghum (Kebrom, Burson et al. 2006), where light is sensed by phytochromes. Mutants of *phyB* increase the *SbTB1* expression that will reduce branching in sorghum (Kebrom, Burson et al. 2006).

Rhizomatousness

Rhizomes are subterranean stems that grow diageotropically (i.e. perpendicular to the force of gravity). They develop either from axillary buds at lower nodes similar to tillers, or from adventitious buds on specialized creeping roots (Gizmawy, Kigel et al. 1985). Rhizomes are a major mechanism of vegetative reproduction and dispersal in many perennial grasses, making some of them noxious weeds. Rhizomatous plants such as bermudagrass (*Cynodon dactylon* L. Pers.) and Johnsongrass (*Sorghum halepense* L. Pers.) were first introduced to the US as promising forage crops and to control soil erosion, but their invasiveness and aggressiveness from rhizomes have made them problematic weeds. Controlling these and many other weeds can be either costly or difficult. For example, there is currently no means to eradicate Johnsongrass from sorghum fields since these two grasses are closely related. Further, hybridization between *S. bicolor* and *S. halepense,* has worsened the situation and may have increased weediness (Morrell, Williams-Coplin et al. 2005).

Although there is much concern about the weediness and problems caused by rhizomes, rhizomes are valuable assets for many perennial forage and turf grasses for continuous

productivity and erosion control. Most of our major row crops being annual, breeding for perenniality has recently attracted attention to lessen soil erosion and improve environmental stewardship by agriculture (Cox, Bender et al. 2002). In many developing countries where most farms are small and family owned, growing perennial plants might be particular attractive, being less time-consuming and inexpensive. Rhizomes, as an essential component of perennial plants, will be beneficial to breeding for perenniality by introgressing rhizome-specific genes into annual plants (Sacks, Dhanapala et al. 2006). Increasing demand for biofuel from chemical feed stocks makes a strong case to utilize genes for aggressive growth from those weeds and to breed perennial plants suitable for growth on agriculture-degraded land (Tilman, Socolow et al. 2009).

Although the molecular control of rhizomatousness is not adequately studied, some work has been initiated in sorghum (Paterson, Schertz et al. 1995), rice (Hu, Tao et al. 2003; Hu, Wang et al. 2011) and maize (Westerbergh and Doebley 2004). QTLs for rhizomatousness fall largely in corresponding genomic regions of sorghum and rice (Hu, Tao et al. 2003), while in a few cases, QTLs responsible for rhizomatous fall in same genomic regions in all three species (Westerbergh and Doebley 2004). This result indicates that genes and genetic pathways for this trait might be conserved in the grass family, so that genes controlling rhizomatousness may also be extrapolated to many other species. That only a small amount of phenotypic variance can be explained by genetic factors also suggests that rhizomatousness is largely affected by the environment and displays low heritability (Paterson, Schertz et al. 1995; Westerbergh and Doebley 2004).

Rhizomes and tillers are developmentally related, since both develop from the axillary buds at the lowest nodes of the plant. In view of this, it was no surprise that some genomic regions controlled both traits (Paterson, Schertz et al. 1995), possibly due to pleotropic effects

(Westerbergh and Doebley 2004), and that similar sets of genes function in both tissues (Jang, Kamps et al. 2006). In spite of their morphological similarities, rhizomes and tillers are physiologically different. Different sets of genes determine the divergent development of tillers and rhizomes and their physiological differences. Above-ground tillers produces inflorescences and seeds, and are subject to senescence, while rhizomes can store and allocate nutrients for perennial growth under poor conditions even at the expense of seeds in temperate regions, facilitating overwintering and rapid growth in the next season.

Hormonal control of rhizomatousness is similar to that already known to regulate axillary meristems (McSteen 2009). Studies have shown that auxin and cytokinin are essential regulators for rhizome development. Similar to tillers, auxin is indispensable in rhizome induction (Kapoor and Rao 2006). Cooperation of various hormones, such as auxin and cytokinin, determines the behavior of rhizomes during their life cycle. For instance, when rhizomes are dormant at the end of autumn, the level of auxin is low and cytokinin is high. In spring and summer when rhizome development is active, the level of auxin is high and cytokinin is low (Maslova, Tabalenkova et al. 2007).

In partial conclusion, discovering QTLs, genes, biochemical pathways, and hormonal control of rhizomatousness will shed new light upon plant growth regulation. Elucidating growth regulators of rhizomes may advance weed control even in closely related plants, such as Johnsongrass in sorghum. Knowledge of rhizomatousness may also benefit the improvement of perennial and turf grasses, and introducing rhizome-specific genes to annual crops may facilitate breeding for perenniality of biomass feedstock on marginal lands. Therefore, extensive and inter-disciplinary studies are required to fulfill this long-term goal.

In summary, there has been progress in studying the genetic determinants of different aspects of plant architecture using different methods in the past decades. Characterizing genes and their biochemical pathways will improve understanding of both conserved mechanisms and the distinct growth habit among plants, to better unravel the evolutionary fate and domestication processes, and facilitate a wide range of applications, such as increasing yield, regulating plant growth, and breeding for perenniality.

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Table 1.1: Genes affecting axillary meristem initiation.

Gene product	**Arabidopsis**	**Rice**	**Maize**
GRAS transcription factor	*LAS* (AT1G55580)	*MOC1* (Os06g0610300)	-
Basic helix-loop-helix transcription factor	*ROX*	*LAX* (Os01g0831000)	*BA1* (GRMZM2G397518)
HD ZIP transcription factor	*REV* (AT5G60690)	*OsHB3* (Os12g0612700)	-
NAC transcription factor	*CUC1,2,3* (AT3G15170, AT5G53950, AT1G76420)	*OsTIL1*/*OsNAC2* (Os04g0460600)	*ZmCUC3* (GRMZM2G009892)

Table 1.2: Genes affecting axillary meristem outgrowth.

Gene product	**Arabdipsis**	**Rice**	**Maize**
P450-type enzyme	*MAX1* (AT2G26170,)		
F-box LRR family	*MAX2* (AT2G42620)	*D3* (Os06g0154200)	
Carotenoid cleavage dioxygenase 7	*MAX3* (AT2G44990)	*D17*/*HTD1* (Os04g0550600)	
Carotenoid cleavage dioxygenase 8	*MAX4* (At4g32810)	*D10* (Os01g0746400)	
TCP transcription factors	*BRC1* (AT3G18550)	*OsTB1* (Os03g0706500)	*TB1* (AC233950.1_FG002)
Iron containing protein		*D27* (Os11g0587000)	
Hydrolase/esterase		*D14* (Os03g0203200)	

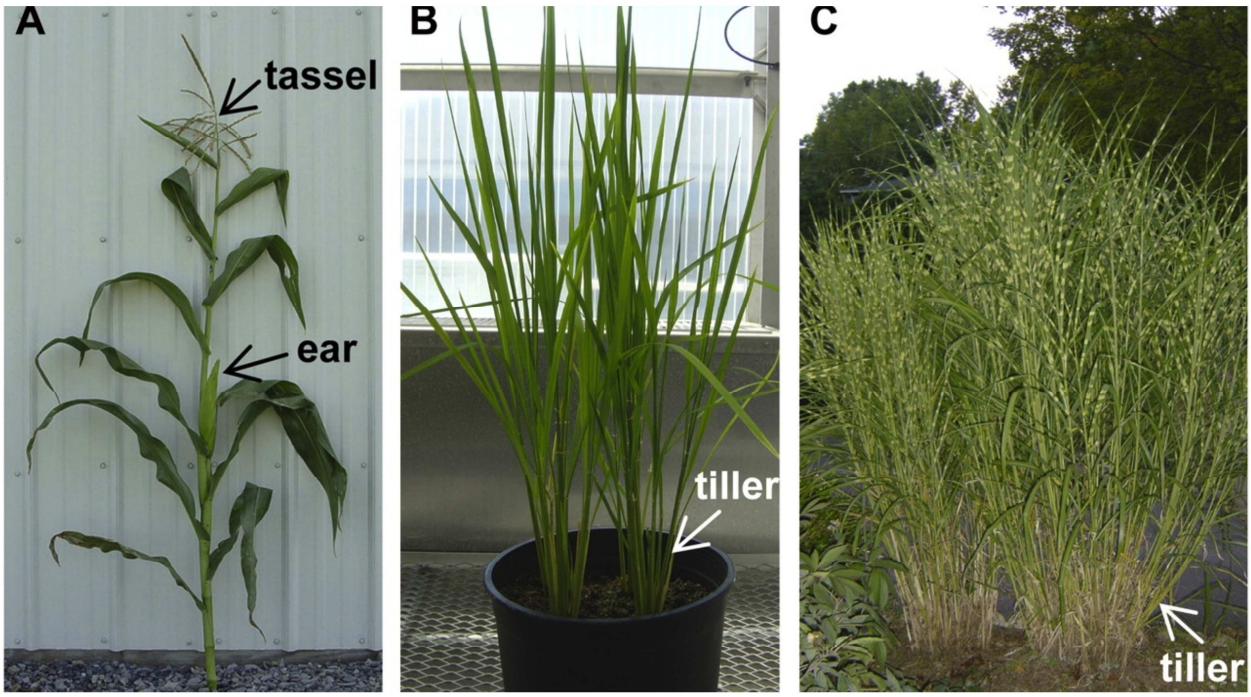

Figure 1.1: Divergent mechanisms of vegetative branching in grasses (adapted from Mcsteen, 2009). A, maize only has a single tiller; B, rice has tillers initiated at early developmental stages; C, Miscanthus has abundant tillers and rhizomes.

CHAPTER 2

GENETIC ANALYSIS OF RECOMBINANT INBRED LINES FOR *SORGHUM BICOLOR* × *SORGHUM PROPINQUUM*[1]

[1] Kong, W, Jin, H, and Franks, CD, et al. 2013. *G3: Genes*| *Genomes*| *Genetics*. 3: 101-108.

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Abstract

We describe a recombinant inbred line (RIL) population of 161 F5 genotypes for the widest euploid cross that can be made to cultivated sorghum (*Sorghum bicolor*) using conventional techniques, *S. bicolor* × *S. propinquum*, that segregates for many traits related to plant architecture, growth and development, reproduction, and life history. The genetic map of the *S. bicolor* × *S. propinquum* RILs contains 141 loci on 10 linkage groups collectively spanning 773.1 cM. Although the genetic map has DNA marker density well-suited to quantitative trait loci mapping and samples most of the genome, our previous observations that sorghum pericentromeric heterochromatin is recalcitrant to recombination is highlighted by the finding that the vast majority of recombination in sorghum is concentrated in small regions of euchromatin that are distal to most chromosomes. The advancement of the RIL population in an environment to which the *S. bicolor* parent was well adapted (indeed bred for) but the *S. propinquum* parent was not largely eliminated an allele for short-day flowering that confounded many other traits, for example, permitting us to map new quantitative trait loci for flowering that previously eluded detection. Additional recombination that has accrued in the development of this RIL population also may have improved resolution of apices of heterozygote excess, accounting for their greater abundance in the F5 than the F2 generation. The *S. bicolor* × *S. propinquum* RIL population offers advantages over early generation populations that will shed new light on genetic, environmental, and physiological/biochemical factors that regulate plant growth and development.

Introduction

As a botanical and genomic model for grasses, *Sorghum bicolor* L. Moench. (sorghum), a native of tropical Africa that is the most drought-resistant of the world's top five cereal crops, is

a logical complement to the largely sequenced genome of rice (*Oryza*). Sorghum has biochemical and morphological specializations to improve carbon assimilation at high temperatures (C4 photosynthesis), whereas rice uses C3 photosynthesis more typical of temperate grasses. Like rice, the most recent genome duplication in sorghum appears to be ~70 million years ago (Paterson, Bowers et al. 2004) simplifying its comparative and functional genomics. With a high-quality DNA sequence (Paterson, Bowers et al. 2009), the ~740 megabase pair sorghum genome is of high value for better understanding the genome of maize (Schnable, Ware et al. 2009) and in particular the impact of an ancient tetraploidy in maize shortly after its lineage diverged from that of sorghum (Swigonova, Lai et al. 2004). Sorghum is of particular importance as a diploid model for the Saccharinae clade of grasses that includes recently formed complex polyploids such as Saccharum (sugarcane, currently the world's no. 1 biofuel crop), and Miscanthus, among the greatest-yielding of biomass crops in the U.S. Midwest (Heaton, Dohleman et al. 2008). Each of these polyploids share substantial genetic colinearity and synteny with sorghum (Ming, Liu et al. 1998; Kim, Zhang et al. 2012), and Saccharum quantitative trait loci (QTL) often show positional correspondence to those of sorghum (Ming, Liu et al. 2001; Ming, Del Monte et al. 2002). One of the few crops suited to all proposed approaches for renewable fuel production. i.e., from starch, sugar, and/or cellulose, sorghum itself is presently the no. 2 U.S. source of fuel ethanol from grain (after maize, and is a promising cellulosic biofuel crop (Rooney, Blumenthal et al. 2007).

Sorghum bicolor × *Sorghum propinquum* is thought to be the widest euploid cross that can be made with the cultigen (*S. bicolor*) by conventional means, and interspecific populations from these species offer opportunities to genetically dissect a wide range of traits related to plant domestication and crop productivity, some of which have begun to receive attention (Chittenden,

Schertz et al. 1994; Lin, Schertz et al. 1995; Paterson, Lin et al. 1995; Paterson, Schertz et al. 1995; Lin, Zhu et al. 1999; Hu, Tao et al. 2003; Feltus, Hart et al. 2006). The opportunities offered by comparison of *S. bicolor* and *S. propinquum* have led to much effort to develop genomics resources, including a detailed genetic map (Chittenden, Schertz et al. 1994; Bowers, Abbey et al. 2003), bacterial artificial chromosome-based physical maps for both species (Lin, Zhu et al. 1999; Draye, Lin et al. 2001; Bowers, Arias et al. 2005), expressed sequence tag (EST) resources (Pratt, Liang et al. 2005), and a genome sequence (Paterson, Bowers et al. 2009).

Among many other aspects of growth and development, *S. bicolor* and *S. propinquum* differ in characteristics related to perenniality, a life history strategy for which the Sorghum genus has become a model (Paterson, Schertz et al. 1995; Hu, Tao et al. 2003; Jang, Kamps et al. 2009). Both consideration of how to expand agriculture to provide plant biomass for production of fuels or chemical feedstocks (Tilman, Socolow et al. 2009), and strategies to rebalance food production with preservation of ecological capital (Glover, Reganold et al. 2010), focus heavily on perenniality. Perenniality may also be a curse—*Sorghum halepense*, a wild perennial polyploid resulting from natural hybridization between *S. bicolor* and *S. propinquum*, finds occasional use as forage and even food (seed/flour) but is most noted as one of the world's most noxious weeds, having spread from its west Asian center of diversity across much of Asia, Africa, Europe, North and South America, and Australia. Demonstration that most genes responsible for variations in size and number in *Sorghum* and *Oryza* of an important perennation organ, the rhizome, map to corresponding chromosomal locations (Hu, Tao et al. 2003), suggests that information about rhizomatousness from a few models (that are also major crops) may extrapolate broadly to a wide range of taxa.

By single-seed descent from the same *S. bicolor* × *S. propinquum* F2 population used in early-generation genetic analysis (Lin, Schertz et al. 1995), we have produced and describe here a recombinant inbred line (RIL) population of 161 F5 genotypes that segregate for a wide range of traits, providing a valuable addition to the genetic resources available for this botanical and genomic model. The genetic control of flowering provides an example of how the RIL population contributes to improved knowledge of trait inheritance.

Materials and Methods

Genotyping and data analysis

The mapping population comprised 161 F5 recombinant inbred lines derived by selfing of single F2 plants described previously (Lin, Schertz et al. 1995), from a controlled cross between single plants of *S. bicolor* BTx623, and *S. propinquum* (unnamed accession). Leaf samples were frozen at -80°C and lyophilized for 48 hours. Genomic DNA was extracted from the lyophilized leaf sample based on Aljanabi et al (Aljanabi, Forget et al. 1999). PCR reactions for SSR analysis were carried out under standard conditions for all primer pairs using 1 U Taq polymerase with 10X PCR buffer (100 mM Tris-HCl at pH 9, 500 mM KCl, and 15 mM MgCl2), 2 mM dNTP, 3 mM MgCl2, 0.2 mM of each primer, and 20 ng of DNA template with a final reaction volume of 10 mL. The thermo-cycling was performed with the following program: (1) Preheat at 95°C for 3 minutes, (2) denaturation at 95°C for 30 seconds, (3) annealing at 65°C for 1 minute (-1°C/ cycle), (4) extension at 72°C for 1 minute, (5) 10 cycles of steps (2) ~ (4), (6) denaturation at 95°C for 30 seconds, (7) annealing at 55°C for 1 minute, (8) extension at 72°C for 1 minute, (9) 32 cycles of steps (6) ~ (7), and (10) final extension at 72°C for 5 minutes. The amplified products were visualized in 10 % polyacrylamide gels with silver staining.

Linkage and QTL analysis

A total of 161 F5 individuals were genotyped. MAPMAKER (Lander, Green et al. 1987) was used for map construction with the data type 'ri self,' which is suitable for the RIL configuration. Heterozygosity in codominant markers was treated as missing data by MAPMAKER because the 'ri self' configuration does not recognize it. Map distances, cM, were calculated using the Kosambi function (Kosambi 1944). Marker loci were grouped by two-point linkage analysis with a logarithm of odds ratio (LOD) threshold of 4.0 and a maximum distance of 30 cM. Local maximum likelihood orders of marker loci were confirmed using the 'ripple' command. The map was drawn using Adobe Illustrator. In 2009, 2010, and 2011, single 1.5-m plots of each RIL were transplanted (2009, 2011) or direct seeded at the University of Georgia Plant Science Farm, Watkinsville, GA, in a completely randomized design. Flowering dates were recorded for the first five flowers per plot. The average of the first five flowering days was calculated in Microsoft Excel. The means of the flowering dates over years were estimated using best linear unbiased prediction with SAS PROC MIXED. Lines, environmental effect, and their interaction were treated as random. The broad sense heritability (H) was calculated using the variance component method ($H = \frac{V_G}{V_G + \frac{V_{GE}}{e} + \frac{V_{residual}}{re}}$). Heritability=60.822/(60.822+ 102.57/3+ 1.5848/3) = 63.66. QTL analysis used composite interval mapping method in Windows QTL Cartographer V2.5_010 (Wang, Basten et al. 2011).

Seed of the RIL population are distributed by the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS), Lubbock, TX (J. Burke).

Results

DNA markers and map construction

A total of 203 SSRs initially were selected and scored, derived from sugarcane ESTs (prefix "CA" or "TC"), previously mapped RFLP probe sequences ["Xcup"(Schloss, Mitchell et al. 2002)], sorghum-sequenced genomic clones ["Xtxp" (Kong, Dong et al. 2000)], sorghum EST sequences ["Xisep" (Ramu, Kassahun et al. 2009)], previously developed SSRs ["Xgap" (Brown, Hopkins et al. 1996)], unpublished SSRs from Agropolis-Cirad- Genoplante ("mSbCIR"), and an unmapped scaffold in the genome sequence. Of those 203 markers, 135 segregating for 141 marker loci were mapped into 10 linkage groups corresponding to the 10 sorghum chromosomes. The remaining markers were excluded due to redundancy (i.e., cosegregation of multiple bands from the same primer) and weak and/or apparent artifactual amplifications. Among the 141 loci mapped in the F5 RILs, there is an average of 9 (5.6%) missing genotypes per locus, with 95% of the loci having less than 29 (18%) missing genotypes. Among 95 loci mapped in the F2 population, there is an average of 25 (6.8%) missing genotypes per locus, with 95% of the loci having less than 103 (27.8%) missing genotypes.

The genetic map of the recombinant inbred lines (RILs) derived from annual *S. bicolor* and perennial *S. propinquum* (Figure 2.1) contains 141 loci on 10 linkage groups collectively spanning 773.1 cM. A total of 35 (24.8%) loci have dominant inheritance, with null alleles from *S. propinquum* at 14 loci and from *S. bicolor* at 21 loci, which is not a significant difference (χ^2=1.4, 1 d.f, P=0.2367). The average interval between consecutive loci is 5.48 cM, ranging from 0.0 cM between cosegregating markers to 25.7 cM in the largest gap (on chromosome 5). Construction of the map used a two-step strategy. First, to minimize ambiguity caused by distorted loci, we constructed a framework map by selecting a subset of clearly scored markers

that also did not deviate significantly from the expected Mendelian ratio (1:1) at $P<10^{-5}$ after Bonferroni correction. To assign linkage groups to chromosomes, we anchored framework markers to physical locations by blasting against the sorghum genome sequence. We then assigned and placed additional markers to the framework at LOD score of $\geq$3.0 and carefully checked for double recombination events in the original scoring data.

Comparison of genetic map to physical positions

Comparison of the genetic map to physical positions of the mapped loci reveals the relationship between genetic distances and physical distances and the physical distribution of markers along the genome. Each marker on the genetic map was aligned to its corresponding physical position by virtue of the published *S. bicolor* genome sequence [Figure 2.1 (Paterson, Bowers et al. 2009)]. The sorghum genome sequence information was given priority in ordering markers that were indistinguishable genetically. Overall, a total of 110 of 141 markers in 10 linkage groups are well aligned to their physical positions. The marker order in the genetic map occasionally deviates from that in the physical map. Distal markers on a linkage group tend to disagree more with the physical map than markers in the middle of a group, presumably due to a lack of flanking markers at the ends of chromosomes. A small number of markers show best matches to sequences that are on different chromosomes from where they map genetically. Factors that may contribute to the discrepancies between the genetic and physical maps include multiple amplifications of paralogous loci; sequence assembly errors; or cryptic structural differences between *S. bicolor* and *S. propinquum*.

The mapped SSR marker loci provide substantial coverage of the genetic map, with the exception of chromosome 6 for which markers only cover the lower one third of the chromosome (Figure 2.1). The unmapped region of this chromosome includes a large

heterochromatic block (about 34 Mb) that contains the *S. propinquum Ma1* allele conferring short-day flowering (Lin, Schertz et al. 1995). There is ample polymorphism between the parental genotypes in this region and we mapped the region in the F2 population (Lin, Schertz et al. 1995). However, the RIL population was advanced in a temperate latitude, and artificial selection has largely eliminated photoperiodic flowering. This selection, together with limited recombination in this heterochromatic region, accounts for it being underpopulated with DNA markers in the RIL map.

Marker distribution is not even along the physical map: markers are concentrated in distal regions and sparse in central regions of the chromosomes. In an extreme case, chromosome 8, a recombinational distance of 4.7 cM spans a physical distance of approximately 46.3 Mb, covering a remarkable 83.4% of the chromosome. This phenomenon is in accordance with our previous observations (Lin, Schertz et al. 1995; Bowers, Arias et al. 2005; Paterson, Bowers et al. 2009) that the sorghum pericentromeric heterochromatin is recalcitrant to recombination, with the vast majority of recombination occurring in the distal euchromatin.

Segregation distortion

In the F5 RILs, all chromosomes except chromosome 7 contain regions with segregation distortion significant at the 5% level (Table 2.1). A total of 14 apices (peak genomic regions) of distortion were found, on chromosome 1 near cM 35.8, chromosome 2 near cM 50.6, chromosome 3 near cM 11.2, 35.0, 66.1, and 84.3; chromosome 4 near cM 77.2, chromosome 5 near cM 0.0 and 60.3; chromosome 6 near cM 0.0; chromosome 8 near cM 39.5; chromosome 9 near cM 26.5 and 37.7; and chromosome 10 near cM 88.7. All regions showed enrichment for *S. bicolor* alleles. Other than the chromosome 6 region under selection for day-neutral flowering, the most striking case of segregation distortion was on chromosome 1—the apex of this

distortion was near the locus Xcup24 with a segregation ratio of 154:3 (homozygous *S. bicolor*: *S. propinquum*). This apex was genetically less than 1 cM from the most extreme case found in the F2 population from which these RILs are derived: the locus CSU507 on LG C (Bowers, Abbey et al. 2003). In a larger set of F2 progeny previously described (Lin, Schertz et al. 1995), we found similarly distorted segregation (203:15) in this region.

We compared the 14 regions of segregation distortion in the F5 RILs to the levels and patterns of segregation found in the F2 population from which these RILs are derived. Because different DNA markers were used in the two studies, this was done by aligning the F2 and F5 genetic maps to their physical locations on the *S. bicolor* genome (Paterson, Bowers et al. 2009). A total of 11 regions of segregation distortion were found in the F2 (Table 2.1). Four of the 11 regions of segregation distortion in the F2 population favored the *S. propinquum* alleles, among which three are no longer distorted in the F5 RILs, and one region near the end of chromosome 2 contains overrepresentation of *S. bicolor* alleles (!) in the F5 RILs. Those regions with overrepresentation of *S. bicolor* alleles in the F2 generally also contain such overabundance in the F5 RILs, albeit a few cases lack nearby DNA markers. However, eight regions showing normal segregation in the F2 showed overabundance of the *S. bicolor* allele in the F5 RILs.

Residual heterozygosity

We compared regions of excess/deficiency of residual heterozygosity in the F5 RILs and the F2 population (Table 2.2). In the F2, 8 regions show excess and 2 show deficiency of heterozygotes. All except two of these also show segregation distortion. In the F5, much higher homozygosity makes it difficult to distinguish heterozygote deficiency with statistical significance but 28 regions show excess, 7 (25%) of which also show segregation distortion. In the F2, the regions showing excess are all small (diagnosed by only 1 marker each), however a

large region of chr. 1 shows deficiency of heterozygotes. In the F5, there are 3 large regions showing heterozygote excess in chromosome 4, 5, and 7 respectively.

Initial QTL mapping

To explore the merit of the RIL population for QTL mapping, we focused on flowering, a trait associated with the tropical origin of *S. propinquum* that had a large confounding effect on many traits in F2 QTL mapping. In the RIL population, near-homozygosity for the *S. bicolor* allele along the salient portion of chromosome 6 reveals that we have largely eliminated genotypes with short-day flowering alleles from *S. propinquum*. A total of three flowering QTL met a LOD threshold of 2.61 based on 1000 permutation tests on chromosomes 4, 8, and 9 (Figure 2.1, Table 2.3). The chromosome 9 QTL found here closely overlaps one found in the F2 generation (Lin, Schertz et al. 1995), which also overlaps a QTL found in several other sorghum populations (Feltus, Hart et al. 2006; Mace and Jordan 2011). The chromosome 8 QTL also closely corresponds to one found in the BTx623 × IS3620c cross (Brown, Klein et al. 2006), and the *S. propinquum* allele confers early flowering, accounting for the transgressants we observed in F2 and F5. The chromosome 4 QTL is newly discovered in this population, perhaps "unmasked" as a result of removing short-day flowering but is in a region in which flowering QTL have been reported previously (Mace and Jordan 2011). Indeed, it shows a "double peak" that may indicate the actions of two nearby genes although we presently infer only a single likelihood interval with statistical confidence. Although a previously reported QTL on chromosome 2 (Lin, Schertz et al. 1995) did not reach statistical significance here, there was subthreshold evidence of it (LOD ~1) in the vicinity that it was previously mapped to.

Discussion

The *S. bicolor* x *S. propinquum* RIL population offers advantages over early-generation populations that promises to shed new light on the genetic, environmental, and physiological/biochemical factors that regulate plant growth and development. Dramatic variation in plant architecture, growth and development, reproduction and life histories of the parental species, together with homozygosity of the RILs and the ability to replicate them across a spectrum of natural and/or controlled conditions, makes this population of high potential importance for the discovery and validation of QTLs.

Advancement of the RIL population in a temperate environment (Lubbock, TX) may improve the ability to resolve QTLs for traits that were previously below the significance threshold, also providing a more realistic assessment of variation that is relevant to temperate latitudes. For example, near-homozygosity for the *S. bicolor* allele along the salient portion of chromosome 6 reveals that we have largely eliminated genotypes with short-day flowering, a trait associated with the tropical origin of *S. propinquum* that had a large confounding effect on many traits in F2-based QTL mapping. Eliminating the profound morphophysiological alteration associated with short-day flowering permitted us to identify two flowering QTLs that eluded detection in our previous study with 370 F2 plants (Lin, Schertz et al. 1995), one of which accounted for the observation that a few segregants flowered earlier than the early-flowering parent.

The advancement of the RIL population in an environment to which the *S. bicolor* parent was well adapted (indeed bred for), but the *S. propinquum* parent was not, may have had some undesirable consequences as well. All segregation distortions in the F5 generation involved excesses of *S. bicolor* alleles, while the F2 generation showed similar numbers of cases of *S.*

bicolor and *S. propinquum* excess. This suggests that in addition to the intended removal of short-day flowering, advancement of the population in temperate continental conditions may have caused some inadvertent selection against other traits of *S. propinquum*, a native of southeast Asia that inhabits streamsides and moist places (ANONYMOUS) (zipcodezoo.com/Plants/S/Sorghum_propinquum/#footref_2). While these biases favoring *S. bicolor* alleles may impact the ability to map QTLs in a few regions of the genome, the population still exhibits a wide range of morpho-physiological variations, with individual lines more comparable to one another by virtue of the near-absence of *Ma1*.

Benefiting from several additional cycles of recombination beyond our prior F2 population, comparison of this genetic map to the sorghum physical map and sequence highlight the striking bias in distribution of recombination across the sorghum genome. This is a good news-bad news scenario –relatively small amounts of physical DNA per cM may facilitate genomic analyses in the gene-rich portions of the genome, but large blocks of recombinationally recalcitrant heterochromatin hinder access to other important genes.

Additional recombination that has accrued in the development of this RIL population may have also improved our ability to resolve apices of heterozygote excess, accounting for their greater abundance in F5 than F2, and occurrence in multiple locations on all chromosomes except the one (chr. 6) for which about two-thirds of the physical length has been fixed due to selection against the *S. propinquum* short-day flowering allele. A remarkably high 28 apices of heterozygote excess, together with rich genetic and genomic tools for these species, may make this an attractive system in which to further dissect the biology underlying interspecific heterozygote advantage.

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Table 2.1: Comparison of regions of segregation distortion between *S. bicolor* (SB) x *S. propinquum* (SP) F5 RIL and F2 populations.

	F5				F2			
Chr.	**marker**	**cM**	**SB:SP**	**Location (Mb)**	**marker**	**SB:SP**	**LG**	**Location**
1	Xcup24	35.8	154:3	13.9	pSB195	203:15	C	14.2
2	CA154181a	50.6	129:24	58.3	pSB101	34:74	B	61.6
2	none near				pSB075	111:35	B	66.1
3	CA199661a	11.2	101:40	0.2	none near		A	
3	CA074959a†	35.0	100:23	3.5- 6.1		N.D.	A	
3	TC48056a†	66.1	86:43	13.8-51.2		N.D.	A	
3	Xcup65	84.3	85:39	55.9		N.D.	A	
3	none near				pSB443b	128:66	A	68.9
4	Xtxp265	77.2	117:37	64.9	none near		F	
4	N.D.				pSB038	34:101	F	14.2
5	mSbCIR329	0.0	134:26	0.2	none near		H	
5	N.D.				pSB064	33:81	H	6.5
5	Xisep1140	60.3	97:52	54.8		N.D.	H	
6	Xgap72	0.0	151:3	41.4	pSB095	104:60	D	50.7
6	none near				pSB428a	93:41	D	38.0
6	none near				pSB643a	65:20	D	4.3
7	N.D.				pSB784	19:50	J	5.9
8	mSbCIR240	39.5	109:49	4.5		N.D.	E	
9	CA142735a	26.5	115:46	31.7		N.D.	G	
9	TC59518b†	37.7	105:55	50.2-54.5		N.D.	G	
10	Xcup43	88.7	123:26	59.8	pSB115	124:59	I	60.6

† Physical location not on the corresponding chromosome of the linkage group: apices are estimated by adjacent marker locations.

N.D. No distortion (not significantly different from 1:1 segregation)

Table 2.2: Comparison of regions showing over-/under-representations of residual heterozygosity between *S. bicolor* x *S. propinquum* F5 RIL and F2 populations.

	F5				F2			
Chr.	**marker**	**cM**	**H/(SB+SP)**	**Location (Mb)**	**marker**	**H/(SB+SP)**	**LG**	**Location (Mb)**
1					pSB102	193:123	C	3.7
1	CA226478a	5.4	21:140	1.8				
1	TC71756a†	97.7	24:135	47.7/50.2-73.4	SHO68	106:244*	C	46.8
2	Xcup67†	12.7	26:133	0.6-2.0				
2	CA296025c	18.2	26:130	2.4				
2	mSbCIR223	37.4	26:131	4.7				
2	Xcup63	51.6	25:136	59.1				
2					pSB101	206:108	B	61.6
2					pSB077	211:103	B	70.0
3	CA152937a	50.2	24:132	7.1				
3	TC48056a†	66.1	28:129	13.8-51.1				
3	TC69429a†	93.4	21:135	55.9-end				
3					pSB443b	81:194*	A	70.0
4	Xcup61†	12.5	36:120	1.5-5.1				
4	Xisep0203	42.5	26:123	10.0				
5	Xtxp065	13.5	23:131	1.9				
5					pSB064	191:114	H	6.5
5	S14_284514_ag17†	52.1	41:116	13.5-42.0				
5	CA100232a	55.6	31:130	54.2				
6					pSB643a	178:85	D	4.3
6					pSB140	221:104	D	52.4
6	Xtxp057	26.4	22:134	5.7				
6					pSB487	194:113	D	60.1
7	Xtxp040	0	28:129	0.9				
7					pSB784	127:69	J	5.9
7	Xtxp278	32.7	26:129	51.1				
7	mSbCIR300	45.6	24:137	5.8				
7	Xisep0829	47.8	31:122	5.9				
8	Xtxp047	29.6	31:126	3.0				
8	CA166256a	51.2	23:137	5.3				
8	Xtxp321	68.1	22:134	5.1				
9	TC50663d	0	20:120	3.0				
9	TC65153a	43.3	23:114	54.4				
9	Xgap206	63.6	21:137	59.2				
10	Xcup49	0	30:130	0.2				
10	CA217392a	26.8	21:132	1.6				
10	CA191677a	81.9	21:139	5.9				

†Physical location not on the corresponding chromosome of the linkage group: apices are estimated by adjacent marker locations.

* Deficiency of heterozygotes (all other cases are heterozygote excess).

Table 2.3: Biometric parameters of QTLs for days to first flower in the *S. bicolor* x *S. propinquum* RILs.

Chr.	LOD	a*	R2	Start (Mb)**	End (Mb)**	Population/study (if not herein)
2	1	-1.2	0.028	60.5	77.9	
2	4.67	-6.7	0.083	61.6	66.1	*S. bicolor x S. propinquum F2* (Lin et al. 1995a)
4(a)	3.0	-1.88	0.094	1.5	5.1	
4(b)	3.5	-2.01	0.108	5.4	10.0	
8	2.8	1.64	0.072	50.5	51.9	
8	5.5	***	0.134	50.5	55.5	*S. bicolor BTx623 x IS3620C* (Brown et al. 2006)
9	4.2	-2.14	0.114	50.2	54.5	
9	2.53	-10.5	0.042	8.1	57.0	*S. bicolor x S. propinquum F2* (Lin et al. 1995a)
9	7.7	-	0.195	****	59.1	*S. bicolor BTx623 x IS3620C* (Feltus et al. 2006)

*Additive effect, calculated as *S. bicolor* BTx623 – other (*S. propinquum* or IS3620C as appropriate). To match this system, values reported in (Lin et al. 1995a) were multiplied by -1.

**Based on flanking DNA marker locations in the published genome sequence (Paterson, Bowers et al. 2009).

***Corresponding values not reported.

****Only a single nearby marker could be definitively mapped to the genome sequence, span of interval uncertain.

Figure 2.1: Genetic map of the *S. bicolor* × *S. propinquum* RILs. For each linkage group, genetic distances are shown on the right in Kosambi centimorgans; their corresponding physical chromosomes (from the current genome assembly, as cited) are shown on the left. Markers whose physical positions are unable to be located to their corresponding chromosomes are indicated by †; markers whose genetic orders disagree with their physical positions are indicated by *. Bar and whisker plots on chromosomes 4, 8, and 9 indicate 1- and 2-LOD likelihood intervals for flowering QTL described in the text, with tick marks indicating likelihood peaks.

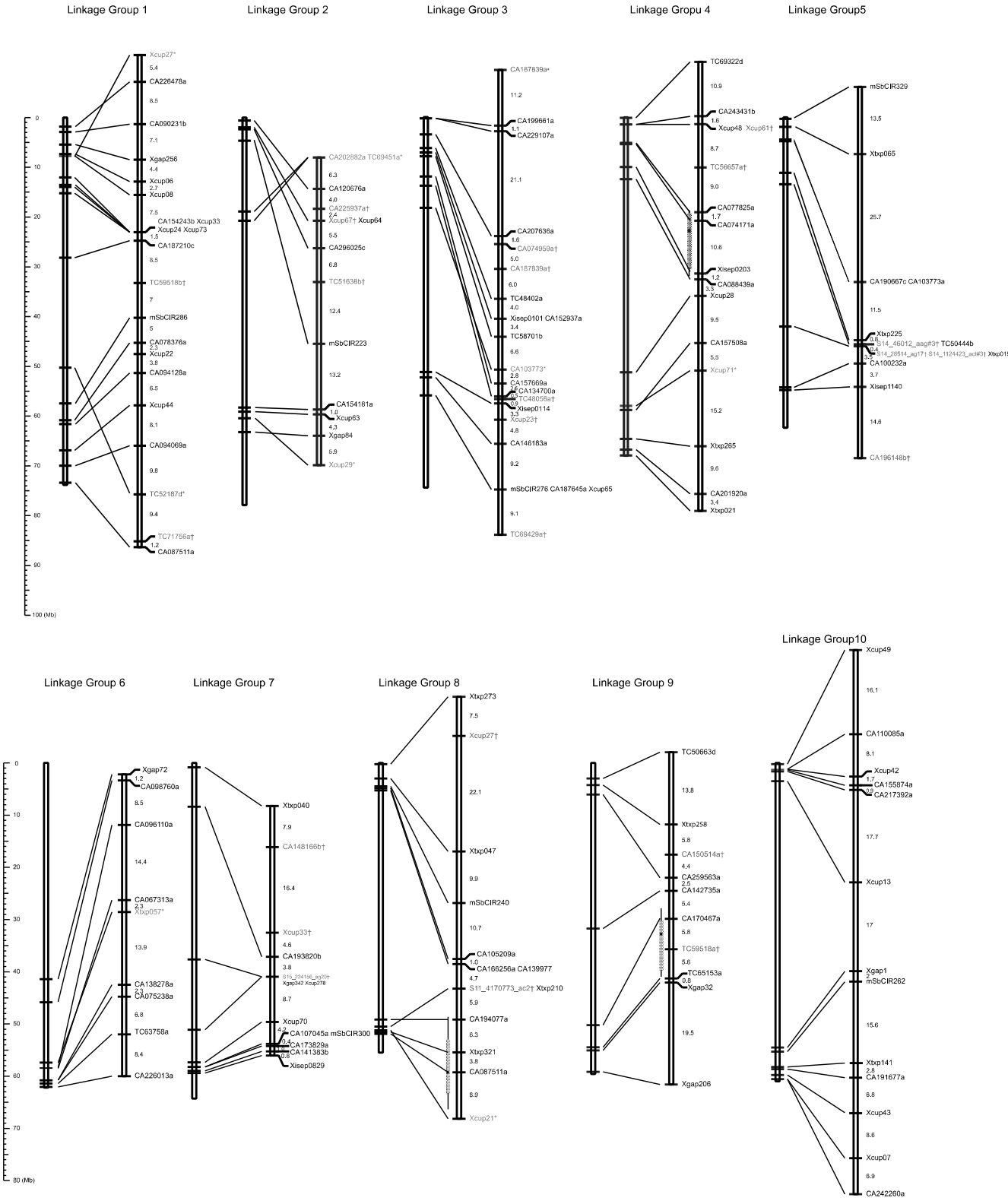
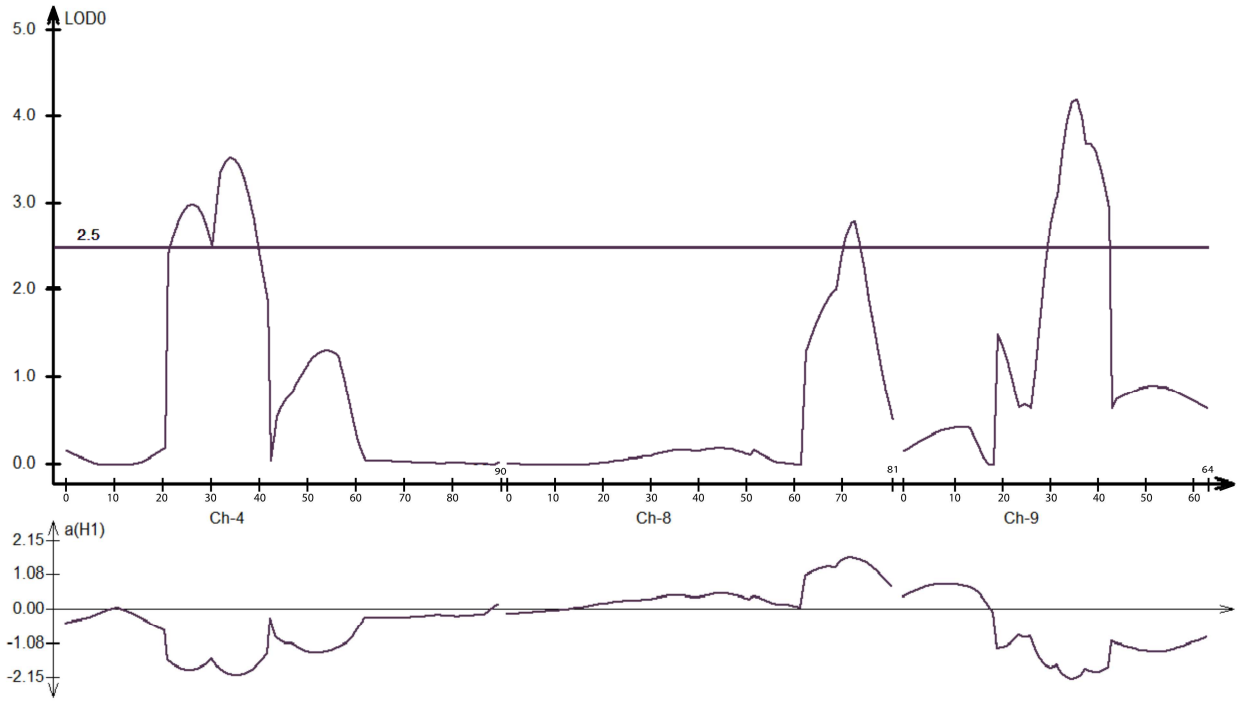

Linkage Group 1
Linkage Group 2
Linkage Group 3
Linkage Gropu 4
Linkage Group5
Linkage Group 6
Linkage Group 7
Linkage Group 8
Linkage Group 9
Linkage Group10
100 (Mb)
80 (Mb)

Figure 2.2: QTLs for days to first flower in the *S. bicolor* x *S. propinquum* RILs. Upper plot represents QTL likelihood (LOD scores) at the indicated cM locations on chromosomes 4, 8, and 9. Lower plot indicates additive effect of an allele substitution at the indicated cM locations, calculated based on flowering times associated with *S. bicolor* minus *S. propinquum* alleles (so positive value indicates earliness associated with the late-flowering *S. propinquum* parent).


LOD0
5.0
4.0
3.0
2.5
2.0
1.0
0.0
0 10 20 30 40 50 60 70 80 90 0 10 20 30 40 50 60 70 81 0 10 20 30 40 50 60 64
Ch-4
Ch-8
Ch-9
a(H1)
2.15
1.08
0.00
-1.08
-2.15

CHAPTER 3

QUANTITATIVE TRAIT ANALYSIS OF VEGETATIVE BRANCHING OF RECOMBINANT INBRED LINES FOR *SORGHUM BICOLOR* × *S. PROPINQUUM*[2]

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Abstract

We describe a three-year study to identify quantitative trait loci (QTL) for plant vegetative branching in a recombinant inbred line (RIL) population of 161 genotypes derived from two morphologically distinct parents, *S. bicolor* × *S. propinquum.* We introduce a phenotypic evaluation system quantifying vegetative branching based on their morphological positions and physiological status. Different sets of QTLs for different levels of vegetative branching were identified. QTLs discovered on chromosomes 1, 3, 7 and 8 affect multiple vegetative variables, suggesting that these regions may contain genes that control general axillary meristem initiation. Other regions that only control one vegetative branching trait could contain genes that control the divergent development of different levels of vegetative branching. We conduct a regression analysis to investigate the relationship between vegetative branching patterns and dry biomass, and conclude that mature tillers and immature (i.e. non-floral) secondary branches show consistent correlation with dry biomass. Among 10 branching-related genes from rice for which we found sorghum orthologs, two (20%) are in syntenic blocks within QTL likelihood intervals, suggesting that they may be candidates for causal elements. Unraveling genetic determinants for plant vegetative branching that are important to food and biofuel productivity may shed new light upon understanding the deterministic development of plants, and designing optimized genotypes for sustainable food and cellulosic biomass production.

Introduction

Plant architecture is determined by the sizes and shapes of plant organs, and patterns of above-ground vegetative branching and underground growth by roots and rhizomes (subterranean stems). Plant architecture decides the dispositions of vegetative organs that capture

light, and the synchrony of inflorescence and seed development that are important factors for grain production. The temporal and spatial development of axillary buds is believed to be largely genetically controlled (Wang and Li 2006; Doust 2007). Therefore, plant architecture frequently contributes to classification of different genotypes into taxa and genera. On the other hand, environmental factors such as density, humidity, temperature and nutrition allow those vegetative organs to achieve a high level of plasticity, making the body plan of a single species variable.

Modifying plant architecture to better suit human uses is an inevitable process during plant domestication. In most of our major crops, the numbers of tillers and axillary branches were significantly reduced during domestication to favor genotypes that were easy to harvest – whether consciously or unconsciously. Grain crops such as sorghum, maize, and millet (the Panicoideae subfamily) have strong apical dominance, while grain crops such as rice and wheat have multiple and synchronized tillers that bear inflorescences to improve seed production (Doust 2007). On the contrary, forage crops such as turf grasses, and many wild plants, have experienced selection for bushiness to produce abundant tillers and rhizomes for perennial growth and herbivory resistance.

Plant vegetative branching is a developmentally important trait, which can be classified based on different positions and magnitudes of maturity. Tillers, formed at a few basal nodes of plants, are important elements for sink capacity of grain crops. Mature tillers produce adventitious roots that specifically supply their development rather than obtaining nutrients from the main stalk (Welker, Briske et al. 1987). Axillary branches are formed in the leaf axils either on the tillers or on previously formed axillary branches, contributing to a bushy architecture. In modern grain crops, development of axillary branches is usually suppressed under genetic and

hormonal control (McSteen 2009). However, maize is an exception with its 'ears' being axillary branches. Bushy architecture resulting from outgrowth of axillary meristems may also be means for increasing cellulosic biomass production, a hypothesis that we will investigate.

The physiological status of vegetative branching is also of importance. For example, plants indigenous to tropical areas, when move to temperate areas, may produce late emerging-tillers and branches. Limited by the climate in temperate areas, those tillers may be either fruitless for grain production of annuals or futile or even harmful resource expenditures for biomass production of perennials that require a balance between single-season and overall-life-time production.

Unraveling the genetic determinants of plant architecture may accelerate development of optimized genotypes for human uses. For example, continued effort to enhance crop grain yield may focus on developing optimized plant architecture rather than simply reducing the number of tillers and axillary branches (Jiao, Wang et al. 2010). Genes responsible for "bushy architecture" may be of practical importance for some plants suitable for biomass production, such as sugarcane and Miscanthus. Identifying genes and discovering genetic pathways responsible for axillary meristem initiation and outgrowth has been a fertile field in tomato (Groot, Keizer et al. 1994), rice (Komatsu, Maekawa et al. 2003; Li, Qian et al. 2003; Takeda, Suwa et al. 2003), Arabidopsis (Sorefan, Booker et al. 2003), maize (Doebley, Stec et al. 1997; Gallavotti, Zhao et al. 2004), pea, petunia (Simons, Napoli et al. 2007) and barley (Dabbert, Okagaki et al. 2009; Dabbert, Okagaki et al. 2010). There is also growing insight into hormonal regulation of vegetative branching (McSteen 2009) : auxin and cytokinin have long been known to affect vegetative branching (Leyser 2003; Leyser 2006; Shani, Yanai et al. 2006; Kyozuka 2007), and the newly discovered hormone, strigolactone, has increased knowledge of molecules that

influence vegetative branching (Gomez-Roldan, Fermas et al. 2008; Umehara, Hanada et al. 2008; Waldie, Hayward et al. 2010). A recent study of gene-gene and genotype by environment interactions (Whipple, Kebrom et al. 2011) has also broadened our knowledge of vegetative branching.

Sorghum uses C4 photosynthetic metabolism that is more water efficient and thought to be better adapted to the tropical areas than plants such as rice and wheat that use C3 photosynthesis. The relatively small genome size (~730 Mb) of *Sorghum bicolor* among C4 plants has made it a botanical model and a reasonable complement to rice as a C3 model (Paterson, Bowers et al. 2009). Sorghum has rich morphological diversity from naturally occurring variation, divergent artificial selection regimes, and novel variation following formation of polyploid *Sorghum halepense*, making it an excellent plant to study plant architecture. To date, quantitative studies of plant architecture in sorghum have been limited to discovering quantitative loci (QTL) responsible for the number of tillers (Lin, Schertz et al. 1995; Paterson, Schertz et al. 1995; Hart, Schertz et al. 2001; Murray, Rooney et al. 2008; Murray, Sharma et al. 2008; Shiringani, Frisch et al. 2010). We are aware of no research in sorghum and little in other species, that has focused on identifying QTLs that influence different vegetative branching patterns either in sorghum or other species (Doust, Devos et al. 2004; Doust and Kellogg 2006).

We report a quantitative trait study to discover genomic regions that underlie different vegetative branching traits based on morphological positions and physiological status in sorghum. A cross between *Sorghum bicolor* and *Sorghum propinquum* (Paterson, Bowers et al. 2009), and their progenies has proved to offer rich information for a wide range of traits (Chittenden, Schertz et al. 1994; Lin, Schertz et al. 1995; Paterson, Schertz et al. 1995; Bowers, Abbey et al.

2003; Hu, Tao et al. 2003; Feltus, Hart et al. 2006). The genetic map of a recombinant inbred line (RIL) population derived from a previous F2 population by single-seed descent has demonstrated its power in an example of detecting flowering QTL (Kong, Jin et al. 2013) and will facilitate quantitative trait loci (QTL) mapping for vegetative branching in this study. Since the RIL population was advanced in a temperate area, eliminating a short-day flowering gene from *S. propinquum* has reduced factors that would otherwise confound development of many traits, and may reveal more QTLs more salient to growth and productivity in temperate regions. Dissecting the morphological and physiological distribution of vegetative branching patterns permits us to distinguish genomic regions that have general control of vegetative branching, from those conferring specific levels or patterns of branching. Better understanding the genetic determinants of different branching patterns and their relationships will promise to shed new light on a variety of applications ranging from plant growth control to breeding for an optimized genotype in different environments.

Materials and Methods

Plant materials

A total of 161 recombinant inbred lines (RIL) derived from a previously described F2 population (Paterson, Schertz et al. 1995) of two morphologically different parents, *Sorghum bicolor* BTx623 and its wild relative, *Sorghum propinquum* (unamed accession) were planted at the University of Georgia Plant Science Farm, Watkinsville, GA, USA, in 2009, 2010 and 2011. Single 1.5-m plots of each RIL were transplanted (on May 20th, 2009 and May 16th, 2011) or directly seeded (May 28th, 2010) in a complete randomized design.

Genetic map

A total of 161 RILs were assayed with 141 SSR markers based on 1:1 segregation ratio. The linkage map constructed using MAPMAKER (Lander, Green et al. 1987) collectively spanned 773.1 cM on 10 linkage groups. The average interval between consecutive loci is 5.48 cM, ranging from 0.0 cM between cosegregating markers to 25.7 cM in the largest gap on chromosome 5. The genetic map was aligned to the physical map by blasting DNA markers sequence to the *S. bicolor* genome sequences to discern the relationship between genetic and physical distances, and the overall distribution of recombination events along the chromosome (Kong, Jin et al. 2013).

Phenotype analysis

Our phenotyping system for vegetative branching integrates the morphological locations and physiological status of each branch; i.e., for each plant, we quantify the number of primary, secondary and tertiary branches based on their morphological locations; and the number of mature floral, immature floral, and vegetative branches based on their physiological status. Primary branches emanate from basal nodes, while secondary branches emanate from primaries, and tertiary branches emanate from secondaries. Higher-order branches, such as quaternary, occurred rarely and were recorded as tertiaries. The total of 9 types of branches, mature primary (M1), mature secondary (M2), mature tertiary (M3), immature primary (IM1), immature secondary (IM2), immature tertiary (IM3), vegetative primary (V1), vegetative secondary (V2), and vegetative tertiary (V3), were recorded for two representative plants from each plot in each year (2009, 2010, 2011). Plants were measured at physiological maturity of most mature primary branches.

Data exploration

To better classify the nine branching measurements and prepare data for effective QTL mapping, we used the following trait combnations. We classify the morphological positions of the branches of each plant based on the number of tillers (TL), which is the sum of primary branches from the basal nodes, and the number of axillary branches (AX), which is the sum of secondary and tertiary branches. To distinguish the physiological maturity of each branch, we measured the numbers of mature (MA), immature (IM), and vegetative branches (VG). To investigate the genetic potential for forming axillary branches, we devised two more measurements, the secondary ratio (SR) and the tertiary ratio (TR). SR is the ratio of the number of secondary branches per node (determined by the product of primaries by the number of nodes, counting nodes on the most mature tiller and assuming that the number of nodes was consistent on each tiller). TR is the ratio of the number of tertiary branches per secondary branch, since the number of nodes on secondary branches was not recorded. Trait means and standard deviations were calculated with the SAS program.

We analyzed the impact of genotype (G), environment (E) and genotype by environment interaction (G×E) using analysis of variance with the type III sums of squares. Different years (from 2009 to 2011) were treated as different environments. Lines, environments, and their interactions were considered random factors. Variance components were used to calculate the broad-sense heritability $H = V_G/(V_G + \frac{V_{G\times E}}{E} + \frac{V_{residual}}{ER})$, in which E is the number of environments and R is the number of replications. Best linear unbiased prediction (BLUP) values were calculated among three years for each branching trait for QTL mapping. The statistical analysis used SAS PROC MIXED.

QTL analysis

Single marker analysis and composite interval mapping (CIM) were performed using Win QTL Cartographer V2.5_010 (Wang, Basten et al. 2011). CIM analysis used the standard model (model 6) with a walking speed of 1 cM and 10 cM window size. Significance thresholds (0.05 experiment-wise) were calculated by 1000 permutation tests.

QTL nomenclature used a system that was described in rice (McCouch, Cho et al. 1997), starting with a 'q', followed by an abbreviation of each trait (TL, AX, MA, IM VG, SR, TR), then the chromosome number, and then a decimal number to differentiate multiple QTLs on the same chromosome.

Biomass analysis

To investigate the relationship between vegetative branching pattern and dry biomass, we conducted a regression study from the 2010 and 2011 data (biomass data was not collected in 2009). Two biomass variables, stem weight (SWT) and leaf weight (LWT), and vegetative branching variables described above were used for regression. A two-step regression method was performed since the nine branching variables showed high correlation coefficients. The first regression was conducted using variables that only confer the positions of vegetative branching, i.e. TL, SecR, which is the number of secondary branches per primary branches, and TR, since these three variables are not highly correlated with each other. Variables that are significant at the level of 0.01 are eligible to enter further regression based on the physiological status. For example, if TL is the only significant variable in the first regression, we subdivide TL into mature primaries (M1), immature primaries (IM1), and vegetative primaries (V1) for another regression study to identify significant components for dry biomass. The second round of

regression analysis used a significance level of 0.05. All statistical analysis used the SAS program.

Toward identification of sorghum genes controlling vegetative branching

A total of ten rice genes (Table 3.5) are known that affect either axillary meristem initiation or outgrowth (Komatsu, Maekawa et al. 2003; Li, Qian et al. 2003; Takeda, Suwa et al. 2003; Zou, Chen et al. 2005; Arite, Iwata et al. 2007; Mao, Ding et al. 2007; Itoh, Hibara et al. 2008; Arite, Umehara et al. 2009; Lin, Wang et al. 2009). We used the "Locus Search" function in the Plant Genome Duplication Database (Lee, Tang et al. 2013) to identify corresponding sorghum genes and investigate their proximity to QTLs for vegetative branching.

Results

Phenotypic distribution of traits

The means and ranges of the seven branching variables of one of the parents, BTx623, and the RILs are shown in Table 3.1. The other parent, *S. propinquum*, is native to tropical or subtropical regions. Growing in a temperate region in this experiment, *S. propinquum* just starts to flower when the temperature reaches the freezing point. Therefore, its vegetative branching patterns were considered not representative and were not used in this analysis.

Two variables indicating the positions of vegetative branches, TL and AX, are correlated with each other ($r=0.5432$, $P<0.0001$). Variables indicating the maturity of branches, MA, IM, and VG are also significantly correlated with each other ($r_{MA:IM}=0.6302$, $r_{MA:VG}=0.2480$, $r_{IM:VG}=0.4759$, $P<0.0001$). RIL means for both positions and maturities (TL, AX, MA, IM and VG) are larger than BTx623 (parental) means. Variables SR and TR are indicators of potential of a plant to form secondary or tertiary branches. Unlike the high positive correlation between AB

and TL, SR and TL are negatively correlated (r=-0.2831, P<0.001), and TR and TL are not significantly correlated (r=0.04378, P=0.26).

It is not surprising that the effect of genotype, environment and genotype by environment interactions are statistically significant (at 0.05) for most traits, since vegetative branching is thought to be among the most plastic of traits (Sultan 2000) (Table 3.2). An exception is the variable MA, where both genotype and environment effects are not significant. The large residual of this trait might be due to variation in the numbers of mature secondary and tertiary branches, which are highly inconsistent among years. Heritability varies widely among different branching traits, implying different levels of plasticity.

QTLs controlling morphological distribution of vegetative branching

A total of four QTLs controlling tillering (on chromosomes 1, 7 (2), and 8) and four QTLs controlling axillary branches (on chromosomes 1 (2), 3, and 8) are significant after 1000 permutation tests (Figure 3.1 and Table 3.3), accounting for 31.96% and 53.60% of phenotypic variation, respectively. For all QTLs detected, *S. propinquum* alleles increase the number of tillers and axillary branches. We are surprised to find that none of these QTLs locate in the same genomic regions, in spite of the morphological similarities of tillers and axillary branches. However, single-marker analysis suggests some overlapping genomic regions controlling both traits on chromosomes 1, 2, 4 and 8. Two 'putative' QTLs for tillering (i.e., that reach LOD 2 but not the higher level indicated by permutation tests) are found on chromosomes 4 and 6. One QTL on chromosome 4 exhibits a positive additive effect for increasing tillering from the *S. bicolor* allele, differing from other QTLs.

Tillering QTLs detected here on chromosomes 1 and 7 overlap with tillering QTLs found in a previous F2 population (Paterson, Schertz et al. 1995), and with QTLs found in other

sorghum populations (Hart, Schertz et al. 2001; Shiringani, Frisch et al. 2010; Mace and Jordan 2011), as revealed using the Comparative Quantitative Trait Locus Database for Saccharinae Grasses (Zhang, Guo et al. 2013). The QTL discovered on chromosome 6 falls in the same genomic region with one found in a sweet sorghum study (Shiringani, Frisch et al. 2010), and the QTL detected on chromosome 8 is closely related to one found in a BTx623 × IS3620C population (Hart, Schertz et al. 2001). Tillering QTLs on chromosomes 1 and 7 fall into high QTL density regions for many other agronomical traits in sorghum (Mace and Jordan 2011).

QTLs controlling physiological maturity of vegetative branching

It is commonly known that not all vegetative branches mature in synchrony. Breeders usually select genotypes with synchronized mature heads to increase seed/grain production of annuals, while selecting genotypes with immature and vegetative tillers or branches for perennial or biomass plants. We believe that physiological maturity is genetically controlled. For example, most modern grain crops are subject to senescence, and all tillers are essentially dead and ready for mechanical harvesting after they become mature. Perennial plants are usually somewhat indeterminate, continually producing moderate numbers of vegetative branches that may also flower throughout their growing season. Our system permits detection of QTLs controlling the numbers of tillers and branches at different physiological status when the primary branches reach maturity, i.e. we differentiate the number of mature, immature, and vegetative branches (Figure 3.1 and Table 3.3). Only one QTL was discovered for the number of mature branches, accounting for 12.07% of phenotypic variance. A total of three and four QTLs for the number of immature and vegetative branches accounted for 39.48% and 23.82% of phenotypic variance, respectively. For all QTLs, *S. propinquum* increased the number of branches. We detected two more ‘putative’ QTLs controlling IM on chromosomes 4 and 5, and two controlling VG on

chromosome 1. The putative QTLs on chromosomes 1 and 4 overlap with TL and AX QTLs and show similar additive effects. This further validates the reliability of the QTLs, albeit not reaching the thresholds of permutation tests.

One overlapping QTL region was found at the interval Xtxp237- Xcup27 on chromosome 8 controlling both MA and IM. Another overlapping interval was on chromosome 3 controlling IM and VG. QTLs found on chromosomes 1, 3, 8 controlling the maturity of vegetative branching also overlap with QTLs underlying tillers and axillary branches, indicating that overlapping sets of genes and biochemical pathways may control axillary meristem initiation related to different levels of vegetative branching.

QTLs controlling the potential for forming axillary branches

Not every node undergoes axillary meristem initiation and outgrowth. Most nodes on the tillers may remain dormant until certain genetic or environmental factors trigger growth at specific developmental stages. For grain crops, secondary and tertiary branches are usually arrested during early developmental stages. In addition, plants may respond differently when they encounter environmental changes such as shading and grazing. We found genetic variation in potential for forming secondary and tertiary branches (SR and TR) by QTL mapping. Three QTLs for SR and three QTLs for TR were identified, explaining 21.08% and 32.26% of phenotypic variance (Figure 3.1 and Table 3.3). Both parents provide alleles for increasing these two traits.

Biomass components related to vegetative branching

A drought tolerant crop, sorghum is an excellent plant for biofuel production without competing for cultivated land for food production. Biomass yield is one of the most important traits in improving sorghum into a biomass-dedicated plant. To investigate effects of branching

on biomass production and identify important components for dry biomass, we performed a regression study using the different branching variables as indicator variables, with leaf biomass and stalk biomass as response variables (Table 3.4). For stalk biomass, mature tillers and immature secondary branches are consistently significant in models. For leaf biomass, mature tillers are significant in both years, while subdividing this variable into a 2nd round of regression yields different sets of significant component variables. This might be due to genotype by environmental interaction or sampling error.

Based on their contributions to stalk biomass, we conducted further QTL analysis for numbers of M1 and IM2. Two QTLs for M1 and three QTLs for IM2 are significant at a LOD score of 2.5, accounting for 11.32% and 25.15% of phenotypic variance (Table 3.3). For all QTLs found, *S. propinquum* alleles increase the number of branches. QTLs on chromosomes 2 and 7 control both traits, indicating that overlapping sets of genes may control these two traits.

Identification of candidate genes in rice

We examined synteny blocks of a total of 10 genes controlling axillary meristem initiation and outgrowth in rice and identified their colinearity in sorghum (Table 3.5) using the Plant Genome Duplication Database (Lee, Tang et al. 2013). The discovered sorghum genes were searched for their relationships with QTL for vegetative branching based on their physical positions. All listed rice genes except *MOC1,* had corresponding sorghum genes. Two sorghum genes that are related to rice genes, *OsTIL1* (Mao, Ding et al. 2007) and *D14* (Arite, Umehara et al. 2009), are within QTL intervals on chromosomes 4 (qIM4.1) and 1 (qAX1.2 and qVG1.2), respectively (Table 3.5). Both *OsTIL1* and *D14* control axillary meristem outgrowth in rice, and *D14* also corresponds to a QTL controlling higher-order branches (qAX1.2), indicating that those corresponding sorghum genes may have the same function.

Discussion

The recombinant inbred line (RIL) population derived from two divergent parents in this study, *S. bicolor* and *S. propinquum,* provides new insights into the genetic control of vegetative branching in sorghum. Replication over multiple environments and little heterozygosity of RILs facilitates the analysis of genotype by environment interactions and precision and validation of QTLs. Advanced in a temperate region (Lubbock, TX), the RIL population improves the ability to discover QTLs relative to a previously-studied F2 population from the same parents (Paterson, Schertz et al. 1995), by eliminating confounding factors that are correlated with short-day flowering from *S. propinquum*. This principle was exemplified by identifying two flowering QTLs (Kong, Jin et al. 2013) that eluded detection in the F2 population (Lin, Schertz et al. 1995). Compared with the previous study (Paterson, Schertz et al. 1995), we validated two previously discovered tillering QTLs and detected two new ones that are validated by independent studies (Hart, Schertz et al. 2001; Shiringani, Frisch et al. 2010). However, eliminating the short-day alleles from *S. propinquum* leads to inadvertent selection towards *S. bicolor* alleles. For example, it is unlikely to detect the tillering QTL on chromosome 6 near the short-day flowering locus that was found in the F2 population (Paterson, Schertz et al. 1995). Segregation distortion due to the selection against short-day flowering might be beneficial to the detection power of QTLs (Xu 2008), but the position and effect of QTL might be affected (Zhang, Wang et al. 2010).

We introduce a phenotyping system to dissect the genetic control of different levels of vegetative branching and demonstrate its efficiency to detect QTLs for each trait in this study. A genomic region on chromosome 3 shows some evidence of QTLs overlapping many traits, including TR, AX, IM and VG. Another “hotspot” is located in the interval Xtxp273 - Xtxp047 on chromosome 8, controlling four vegetative branching traits (qAX8.1, qMA8.1, qIM8.1 and

qSR8.1). Genomic regions on chromosomes 1 and 7 also controlled at least 5 vegetative branching traits. The QTL regions controlling many branching traits support our expectation that different levels of branching may share some common genetic control for axillary meristem initiation. This could be either due to pleotropic effects of single genes in the identified genomic regions, or could suggest high concentrations of different genes in particular chromosomal regions. Another reason for some genomic regions to contain multiple vegetative branching traits could be inter-relationships between traits. For example, since secondary and tertiary branches are mostly immature or vegetative, it may be possible to find a common QTL that controls all of these traits. However, there is also clear evidence that some traits, such as tillering and high-order branching, have degrees of distinct genetic control by showing different sets of QTLs. The additive effect shown by each QTL suggests that alleles increasing vegetative branching are mostly coming from S. *propinquum*, and only rarely from *S. bicolor*.

Vegetative branching is a highly plastic trait, with the effects of genotype, environment and their interactions generally all significant. Large environmental contributions and genotype by environment interactions may also lead to different sets of QTLs, for different vegetative branching traits might differ in plasticity from each other, demonstrated by their heritability. For example, the number of tillers might be more consistent among different environments than higher-order branches, since the latter trait is more likely to respond to changing environments. QTLs for certain vegetative branching trait might be significant while others remain under the threshold level mainly due to the environment. To determine whether the effects of QTLs are caused by different genes or environment requires multi-environment testing, comparison to other populations, and ideally positional cloning genes and testing of gene functions.

A drought-tolerant plant, sorghum is a promising candidate for biomass-dedicated feedstock to be grown in marginal land without competing for land for food (Rooney, Blumenthal et al. 2007). Vegetative branching is an important component for increasing biomass. This study provides guidance for improving vegetative architecture of biomass-dedicated crops. That vegetative branching pattern is differently related to stalk biomass and leaf biomass respectively, suggests separate genetic controls for these two biomass component traits. Although the result is variable, mature tillers and immature secondary branches are consistently correlated to both dry stalk and leaf biomass, implying that efforts to increase these two traits may improve biomass production.

Identification syntenic relationships of rice genes controlling axillary meristem initiation along the sorghum genome may facilitate discovery of corresponding sorghum genes within the QTL interval. With the help of the Plant Genome Duplication Database, all ten characterized rice genes except *MOC1* were related to colinear corresponding sorghum genes. Two sorghum genes, related to rice *OsTIL1* and *D14*, locate within QTL intervals found in this study. Further association and functional analyses may validate theses two candidate genes and elucidate their functions. The rest of the candidate genes do not correspond to sorghum branching QTLs. One gene related to rice *D17* on chromosome 6 may not detect QTL correspondence due to selection against another allele (short-day flowering) in the region. Further, the physical positions of three sorghum genes, in the syntenic blocks with *LAX*, *OsHB3*, and *D10* are located at the distal region of their respective chromosomes, slightly beyond the range of this genetic map—however we see no evidence of QTLs in these regions based on the nearby markers that are mapped.

QTLs for vegetative branching revealed in this study may be valuable in several ways for different sorghum improvement programs. Breeders dedicated to grain sorghum breeding may

utilize this QTL information to further increase the degree of apical dominance and suppress the growth of axillary meristems. On the other hand, breeding for biofuel feedstocks and possibly perennial crops might increase productivity by introducing alleles from *S. propinquum* or other sources and balancing resources at different developmental stages.

The high degree of common genetic control of many traits across Poaceae grasses suggests that identification of specific genes related to elements of plant architecture may have value in diverse contexts, for example, in improvement of a wide range of grain, forage, biomass, and turfgrasses. QTLs identified in this population may also contribute to narrowing down the genomic regions containing underlying genes, and eventually facilitate the positional cloning of genes for vegetative branching in sorghum.

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Table 3.1: Trait values for recombinant inbred lines (RILs) and BTx623 in three years.

Trait	2009			2010			2011		
	BTx623	RILs		BTx623	RILs		BTx623	RILs	
	Mean (SD)	Mean (SD)	Range	Mean (SD)	Mean (SD)	Range	Mean (SD)	Mean (SD)	Range
TL	2.25 (0.95)	14.41 (8.74)	1-45	5.13 (2.22)	16.80 (10.96)	2-61	4.50 (1.27)	7.80 (5.06)	1-33
AX	2.85 (1.27)	19.48 (15.69)	1-121	5.38 (1.73)	36.32 (31.67)	0-185	5.90 (2.60)	25.65 (27.26)	0-171
MA	1.75 (0.54)	4.06 (3.07)	1-16	1.56 (0.86)	6.53 (5.44)	1-35	1.80 (0.63)	5.52 (4.29)	1-29
IM	2.55 (1.61)	19.06 (14.94)	0-119	7.25 (2.74)	38.11(31.49)	0-194	6.20 (3.22)	23.49 (23.95)	0-157
VG	0.80 (0.35)	10.77 (7.50)	0-42	1.69 (1.46)	8.49 (8.12)	0-55	2.40 (2.22)	4.44 (4.65)	0-29
SR	0.12 (0.06)	0.087 (0.056)	0.0067-0.35	0.10 (0.06)	0.11 (0.064)	0-0.38	0.067 (0.02)	0.18 (0.11)	0-0.64
TR	0.07 (0.12)	0.61 (0.63)	0-4.4	0.31 (0.19)	0.72 (0.53)	0-3	0.52 (0.44)	0.77(0.79)	0-4.63

Table 3.2: Trait heritability and variance components based on genotype, year, and genotype by year interaction percentage.

Traits	Rep (Year) (%)	Genotype (%)	Year (%)	Genotype×Year (%)	Residual	Heritability (%)
TL	- NS	19.5 ***	33.8 ***	13.3 ***	33.5	66.0
AX	- NS	16.0 ***	6.6 *	19.4 ***	58.0	49.8
MA	- NS	4.3 NS	1.6 NS	27.8 ***	66.3	17.3
IM	- NS	13.2 ***	13.3 ***	16.2 ***	57.3	47.0
VG	5.6 ***	22.6 ***	14.6	15.9 ***	41.3	64.9
SR	- NS	7.2 *	24.0 ***	19.9 ***	49.0	32.7
TR	1.1 *	34.7 ***	-NS	18.5 ***	45.7	71.6

NS: not significant.

* Significant at 0.05 level.

** Significant at 0.01 level.

*** Significant at 0.001 level.

Rep (Year): replication effect was nested within years.

Table 3.3: QTLs affect vegetative branching in the *S. bicolor* and *S. propinquum* RILs.

Trait	QTL name	Chr	Position	LOD	Additive	R^2	Start (Mb)[a]	End (Mb)
TL	qTL1.1	1	51.8	6.8	-1.49	0.121	28.1	60.8
TL	qTL7.1	7	16.9	3.3	-0.95	0.070	0.9	8.4
TL	qTL7.2	7	32.7	2.8	-0.79	0.045	8.4	58.2
TL	qTL8.1	8	53.2	4.8	-1.02	0.083	4.9	51.5
TL †[b]	qTL4.1	4	62.0	2.3	0.71	0.036	58.8	64.6
TL †	qTL6.1	6	55.4	2.4	-0.75	0.043	60.8	62.1
AX	qAX1.1	1	40.3	6.0	-8.89	0.300	28.2	57.5
AX	qAX1.2	1	68.9	2.5	-2.00	0.059	64.0	70.0
AX	qAX3.1	3	50.2	6.4	-2.89	0.123	6.2	7.8
AX	qAX8.1	8	0.0	3.0	-1.74	0.054	0.2	2.97
SR	qSR3.1	3	66.1	4.5	-0.0045	0.098	13.8	51.2
SR	qSR7.1	7	24.3	3.0	0.0035	0.060	0.9	37.7
SR	qSR8.1	8	7.5	2.5	-0.0032	0.052	0.2	3.0
TR	qTR3.1	3	50.2	12.0	-0.16	0.216	6.2	7.8
TR	qTR5.1	5	51.7	2.8	0.087	0.056	4.8	42.0
TR	qTR9.1	9	29.5	2.7	-0.077	0.050	4.2	54.5
MA	qMA8.1	8	1.0	5.2	-0.12	0.121	0.2	3.0
IM	qIM1.1	1	40.3	3.7	-6.43	0.222	28.2	57.5
IM	qIM3.1	3	50.2	5.4	-2.22	0.100	6.2	7.8
IM	qIM8.1	8	0.0	4.1	-1.75	0.073	0.2	3.0
IM †	qIM4.1	4	56.5	2.2	1.32	0.041	51.2	58.8
IM †	qIM5.1	5	24.5	2.0	-1.94	0.078	0.2	4.5
VG	qVG2.1	2	50.6	3.3	-0.87	0.058	4.7	63.2
VG	qVG3.1	3	50.2	3.3	-0.72	0.060	6.2	7.8
VG	qVG7.1	7	34.7	3.2	-0.68	0.063	8.4	58.3
VG	qVG8.1	8	52.2	5.2	-0.85	0.096	4.5	51.5
VG †	qVG1.1	1	48.8	2.5	-0.80	0.051	28.2	60.8
VG †	qVG1.2	1	67.9	2.4	-0.66	0.050	64.0	66.9
M1		2	56.9	2.6	-0.11	0.06	59.1	63.2
M1		7	41.4	2.5	-0.082	0.053	51.1	58.6
IM2		1	68.9	4.4	-0.68	0.095	64.0	70.0
IM2		2	55.9	3.4	-0.06	0.065	59.1	63.2
IM2		7	41.4	4.7	-0.60	0.092	51.1	58.3

[a] Based on DNA marker locations flanking 1- LOD interval in the published genome sequence (Paterson, Bowers et al. 2009).

[b] †: Significant at a LOD score of 2.0

Table 3.4: Vegetative branching variables related to stalk and leaf biomass.

Response variables	First regression [a]				Second regression [b]					
	Indicator variables			Model R^2	Indicator variables					
	TL	SecR	TR		M1	IM1	V1	M2	IM2	V2
Stalk weight 2010	<0.0001**	0.0012**	0.5359	0.17	0.006**	0.0322*	0.1836	0.5533	<0.0001**	0.2487
Leaf weight 2010	<0.0001**	0.0254	0.2294	0.2	0.0951	0.0265*	0.0276*	-	-	-
Stalk weight 2011	<0.0001**	0.0002**	0.0179	0.3	<0.0001**	0.5647	0.0792	<0.0001	0.0191*	0.0721
Leaf weight 2011	<0.0001**	0.0008**	0.0165	0.36	<0.0001**	0.1318	0.0026**	<0.0001	0.0235*	0.0023*

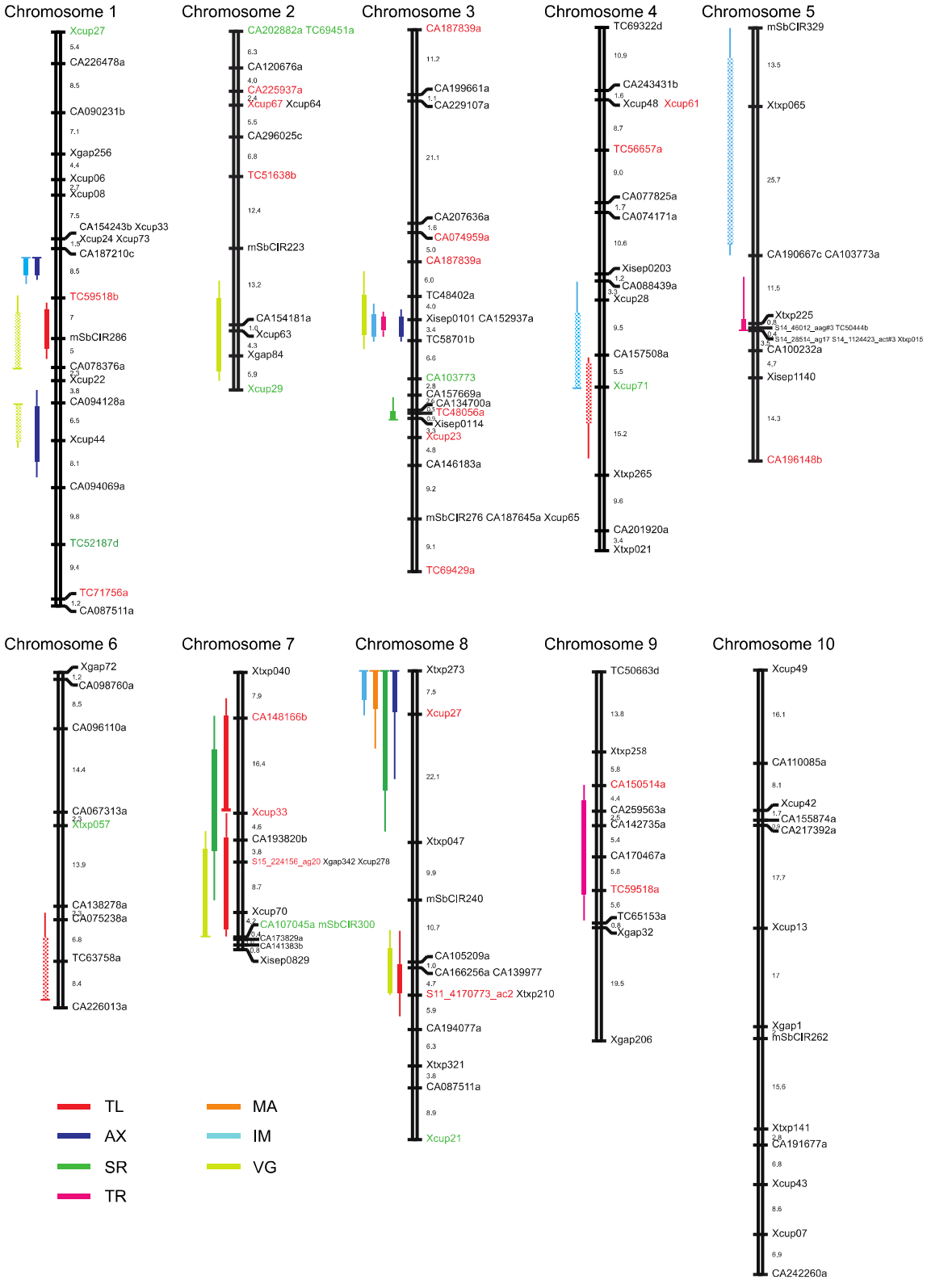
[a] Significant level of the first regression is 0.01

[b] Significant level of the second regression is 0.05

Table 3.5: Genomic positions of candidate sorghum genes that correspond to characterized rice genes controlling axillary meristem initiation and outgrowth.

Gene Name	Rice ID	Sorghum ID	Start	End	RIL QTL
Genes controlling axillary meristem initiation					
MOC1	Os06g0610300	NO synteny			-
LAX PANICLE	Os01g0831000	Sb03g038820	66624442	66623744	No
OsHB3	Os12g0612700	Sb08g021350	52952953	52946784	No
		Sb01g013710	12771371	12776651	No
Genes controlling axillary meristem outgrowth					
OsTIL1/*OsNAC2*	Os04g0460600	Sb04g023990	53666487	53667837	qIM4.1
		Sb06g019010	48600551	48601868	No
D3	Os06g0154200	Sb10g003790	3276753	3278855	No
D17/*HTD1*	Os04g0550600	Sb06g024560	53677260	53679729	No
D10	Os01g0746400	Sb03g034400	62611870	62608453	No
OsTB1	Os03g0706500	SB01g010690	9507199	9506057	No
D27	Os11g0587000	Sb05g022855	55156777	55161632	No
D14	Os03g0203200	Sb01g043630	66780322	66779168	qAX1.2, qVG1.2

Figure 3.1: QTL mapping of vegetative branching in *S. bicolor* × *S. propinquum* RILs. QTLs are shown with 1-lod (solid or dotted) and 2-lod (whiskers) intervals. Solid boxes indicated that the QTL is also significant after 1000 times of permutation test. Dotted boxes are 'putative' QTLs significant only at a LOD score of 2. Markers whose physical locations are unable to be located to their corresponding chromosomes are in red; markers whose genetic orders disagree with their physical locations are in green as detailed elsewhere (Kong, Jin et al. 2013).


Chromosome 1
Xcup27
5.4
CA226478a
8.5
CA090231b
7.1
Xgap256
4.4
Xcup06
2.7
Xcup08
7.5
CA154243b Xcup33
Xcup24 Xcup73
1.5
CA187210c
8.5
TC59518b
7
mSbCIR286
5
CA078376a
2.3
Xcup22
3.8
CA094128a
6.5
Xcup44
8.1
CA094069a
9.8
TC52187d
9.4
TC71756a
1.2
CA087511a
Chromosome 2
CA202882a TC69451a
6.3
CA120676a
4.0
CA225937a
2.4
Xcup67 Xcup64
5.5
CA296025c
6.8
TC51638b
12.4
mSbCIR223
13.2
CA154181a
1.0
Xcup63
4.3
Xgap84
5.9
Xcup29
Chromosome 3
CA187839a
11.2
CA199661a
1.1
CA229107a
21.1
CA207636a
1.6
CA074959a
5.0
CA187839a
6.0
TC48402a
4.0
Xisep0101 CA152937a
3.4
TC58701b
6.6
CA103773
2.8
CA157669a
CA134700a
TC48056a
Xisep0114
3.1
Xcup23
4.8
CA146183a
9.2
mSbCIR276 CA187645a Xcup65
9.1
TC69429a
Chromosome 4
TC69322d
10.9
CA243431b
1.6
Xcup48 Xcup61
8.7
TC56657a
9.0
CA077825a
1.7
CA074171a
10.6
Xisep0203
1.2
CA088439a
3.3
Xcup28
9.5
CA157508a
5.5
Xcup71
15.2
Xtxp265
9.6
CA201920a
3.4
Xtxp021
Chromosome 5
mSbCIR329
13.5
Xtxp065
25.7
CA190667c CA103773a
11.5
Xtxp225
0.8
S14_46012_aag#3 TC50444b
0.5
S14_28514_ag17 S14_1124423_act#3 Xtxp015
3.5
CA100232a
4.7
Xisep1140
14.3
CA196148b
Chromosome 6
Xgap72
1.2
CA098760a
8.5
CA096110a
14.4
CA067313a
2.4
Xtxp057
13.9
CA138278a
2.9
CA075238a
6.8
TC63758a
8.4
CA226013a
Chromosome 7
Xtxp040
7.9
CA148166b
16.4
Xcup33
4.6
CA193820b
3.8
S15_224156_ag20 Xgap342 Xcup278
8.7
Xcup70
4.4
CA107045a mSbCIR300
CA173829a
CA141383b
0.8
Xisep0829
Chromosome 8
Xtxp273
7.5
Xcup27
22.1
Xtxp047
9.9
mSbCIR240
10.7
CA105209a
1.0
CA166256a CA139977
4.7
S11_4170773_ac2 Xtxp210
5.9
CA194077a
6.3
Xtxp321
3.8
CA087511a
8.9
Xcup21
Chromosome 9
TC50663d
13.8
Xtxp258
5.8
CA150514a
4.4
CA259563a
2.5
CA142735a
5.4
CA170467a
5.8
TC59518a
5.6
TC65153a
0.8
Xgap32
19.5
Xgap206
Chromosome 10
Xcup49
16.1
CA110085a
8.1
Xcup42
1.7
CA155874a
2.5
CA217392a
17.7
Xcup13
17
Xgap1
2.3
mSbCIR262
15.6
Xtxp141
3.8
CA191677a
6.8
Xcup43
8.6
Xcup07
6.9
CA242260a
TL
AX
SR
TR
MA
IM
VG

CHAPTER 4

GENETIC ANALYSIS OF RHIZOMATOUSNESS AND ITS COMPARISON TO VEGETATIVE BRANCHING OF RECOMBINANT INBRED LINES FOR *SORGHUM BICOLOR* × *S. PROPINQUUM*[3]

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Abstract

We report a quantitative study to discover the genetic determinants of rhizomatousness, an important trait related to perenniality and invasiveness. A recombinant inbred line (RIL) population of 161 individuals derived from two morphologically distinct parents, *S. bicolor* × *S. propinquum*, segregates for rhizomatousness. We report a total of 11 genomic regions and four consensus regions for rhizomatousness that showed correspondence with previously detected QTL in an F2 population, and with different levels of vegetative branching patterns. In addition to identifying the regions for presence of rhizomatousness, we also report QTLs for the number of rhizomes and the distances of rhizome-derived shoots from the crown that they grew from. As rhizomatousness is a plastic trait that is greatly influenced by environment, consensus regions that overlap with those discovered in the F2 population provide validation of the position and effect of QTLs. Correspondence with regions influencing vegetative branching patterns indicate that some controlling genes and biochemical pathways may be shared by branches and rhizomes during early developmental stages, while genes controlling only one trait may control divergent development of these analogous organs. Manipulation of genes conferring rhizomatousness may provide opportunities for plant growth regulation that will benefit diverse applications: increasing rhizomatousness may promote the productivity and perenniality of many grasses, especially the biomass-dedicated crops; decreasing rhizomatousness may improve grain production and control many noxious weeds.

Introduction

Rhizomes, subterranean stems that grow diageotropically (perpendicular to the force of gravity), develop from axillary buds on the basal portion of seedling shoots (Gizmawy, Kigel et al. 1985). Rhizomes are highly associated with overwintering and regrowth in many perennial

grasses. Perennial and annual plants often have striking differences in their ways of adapting to environment: many annuals utilize seed dispersal, while many perennials develop abundant underground networks to enable vegetative reproduction. The general association of perennial plants with wild species and annual plants with cultivated crops suggest that perennial habit is ancestral to annual habit. Identification of genes underlying rhizomatousness and related the biochemical pathways will contribute to understanding the features of perennialism and their evolutionary story.

Rhizomes are an important component of persistence and spread of many noxious weeds, such as johnsongrass (*Sorghum halepense* L. Pers.), bermudagrass (*Cynodon dactylon* L. Pers.), purple nutsedge (*Cyperus rotundus*) and others. Both johnsongrass and bermudagrass were introduced into the US as promising forage crops. However, their invasiveness makes eradication of these weeds in cultivated land quite costly, or improbable; for example, there is currently no means to control johnsongrass in the sorghum field.

On the contrary, the aggressive nature of rhizomatousness can also become a blessing. Rhizomes are a valuable asset in many forage crops and turf grasses, providing pleasing landscapes and supporting animal grazing. Not only do these crops have economic value, but they also contribute ecosystem services such as preventing soil erosion through the complex underground network of roots and rhizomes. Recently, breeding for dedicated chemical feedstock for biofuel production has been a priority to meet increasing demand for energy caused by population increase and limited fossil fuel resources. The fascinating growth mode of the invasive species is extremely attractive in providing stable production of feedstock in marginal land with little input. Further, a degree of rhizomatousness in modern grain crops may permit

breeding for perenniality, toward production systems which may help prevent soil erosion and improve sustainable grain production (Cox, Bender et al. 2002).

Rhizomes and tillers are developmentally related, both initiated at the lowest node of a plant. Botanically, rhizomes are modified stems (indeed, some primitive plants have rhizomes but not stems so it may be more accurate that stems are modified rhizomes!) and many of the same genes are expressed in each of these tissues (Jang, Kamps et al. 2006). After buds are formed, immediate orientation differentiation of tillers and rhizomes caused by a position gradient determines their different fates. Above-ground tillers produce inflorescences and seeds, and are subject to senescence, while rhizomes can store and allocate nutrients for perennial growth under poor conditions even at the expense of seeds in temperate latitudes, facilitating overwintering and rapid growth early in the next season.

Previous studies have discovered quantitative trait loci (QTL) conferring rhizomatousness in sorghum (Paterson, Schertz et al. 1995), rice (Hu, Tao et al. 2003) and maize (Westerbergh and Doebley 2004). While the maize chromosomes have differentiated greatly from those of sorghum and rice due to maize lineage-specific genome duplication, QTL conferring rhizomatousness still correspond to those of sorghum and/or rice in a few cases (Westerbergh and Doebley 2004).

Despite their morphological similarity, little research has been focused on comparing genomic regions for rhizomes and vegetative branching. Moreover, as a trait that is largely influenced by the environment, rhizomes are best evaluated in multiple conditions. However, prior studies were conducted in F2 populations, limiting the evaluation of this trait to only one environment. Further investigation of genomic regions for rhizomatousness is needed to confirm previously discovered QTLs.

As a botanical model for C4 grasses, the genus *Sorghum* provides numerous resources to study rhizomatousness (Paterson, Schertz et al. 1995; Jang, Kamps et al. 2006; Jang, Kamps et al. 2008) and its comparison to vegetative branching. Morphological diversity occurring during divergent evolution of sorghum relatives both in the wild and under domestication makes it possible for us to create segregating populations, and map genetic determinants of both rhizomatousness and branching. The published ~740 Mb genome sequence (Paterson et al. 2009a) is a valuable resource toward discovering gene functions, biochemical pathways and comparative genetic studies for rhizomatousness and vegetative branching.

In this study, we investigate genomic regions conferring rhizomatousness in a recombinant inbred line (RIL) population derived by single seed descent from a previously described F2 population (Paterson, Schertz et al. 1995). The cross between *S. bicolor* and *S. propinquum* is the widest euploid cross that can be made by conventional methods with the cultigen, and their progenies have provided rich genetic information to study a wide range of traits (Chittenden, Schertz et al. 1994; Lin, Schertz et al. 1995; Paterson, Schertz et al. 1995; Bowers, Abbey et al. 2003; Hu, Tao et al. 2003; Feltus, Hart et al. 2006; Kong, Jin et al. 2013). Evaluation of phenotypic traits such as vegetative branching and rhizomatousness that are highly plastic is advantageous in RIL populations, that can be tested in multiple environments. Discovery of genomic regions responsible for rhizomatousness in this population offers a means to validate the positions and effects of QTLs in the F2 population and facilitate comparisons between rhizomatousness and vegetative branching. Genomic regions discovered from this interspecific RIL population will lay a solid foundation for positional cloning of causal genes, which may either generally control both vegetative branching and rhizomatousness, or control unique patterns of development and metabolism that differentiate these organs. Identification of

genes that are important to rhizomatousness may benefit a wide variety of applications, ranging from regulating plant growth to breeding for perenniality.

Materials and Methods

Plant materials

A total of 161 recombinant inbred lines (RIL) derived from a previously described F2 population (Paterson, Schertz et al. 1995) of two morphologically different parents, *Sorghum bicolor* BTx623 and its wild relative, *S. propinquum* (unamed accession) were planted at the University of Georgia Plant Science Farm, Watkinsville, GA, USA, in 2009, 2010 and 2011. Single 1.5-m plots of each RIL were transplanted (on May 20th, 2009 and May 16th, 2011) or directly seeded (May 28th, 2010) in a completely randomized design.

Phenotypic evaluation

Our system for measuring rhizomatousness was similar to that used in the F2 population of the same cross (Paterson, Schertz et al. 1995). The number of rhizome-derived shoots (RN) was counted for two representative plants in each plot in a 3-year experiment (2009, 2010, 2011). Cases in which we were unable to distinguish rhizome-derived shoots from crown-derived shoots were scored “intermediate”. In addition, we measured the distances from every rhizome-derived shoot found to the center of the crown that it grew from (RD, rhizome distances), if necessary verifying the source crown by digging.

Data analysis

Several data analysis approaches were utilized and compared. First, we treated phenotypic data as qualitative and classified plants into three categories: with rhizomes (Y); no rhizomes (N); and intermediate (I). Fisher’s exact test applied to a contingency table was used to investigate the relationship between rhizome phenotype and each DNA marker genotype.

Statistically significant markers were identified at the level of 0.05. We conducted hypothesis tests for a total of 141 markers. The p-value of 0.05 has a 5% false positive rate, implying that 7 of 141 markers will be false positive if all markers are independent. To set a more stringent false positive level, we accepted a false discovery rate (FDR) of 0.2 to estimate the proportion of rejected null hypotheses that were falsely rejected (Benjamini and Hochberg 1995). To calculate the effect of each significant genomic interval, we chose the most significant marker in the interval. For each of the two genotypes (*S. bicolor, S. propinquum*) at a marker locus, we calculated the ratio of rhizomatousness by summing the number of rhizomatous individuals plus one half the number of intermediate individuals, then dividing by the total number of individuals. Allele effects were calculated as the difference between the *S. bicolor* and *S. propinquum* ratios, divided by two.

We compared the genomic regions for rhizomatousness in the RILs with the previous QTL mapping results from the F2 population (Paterson, Schertz et al. 1995), and with vegetative branching data (Chapter 3), aided by the published sorghum genome sequence (Paterson, Bowers et al. 2009) and the Comparative Saccharinae Genome Resource-QTL database (Zhang, Guo et al. 2013).

QTL mapping

We conducted QTL mapping for the number of rhizomes (RN) using nonparametric interval mapping with the R/qtl package (Broman, Wu et al. 2003), separately for data from each of the three years. We assigned intermediate phenotypes with a value of 0.5. A permutation test was performed to define the LOD threshold. Significant QTLs detected were selected and fitted into multiple-QTL models (Arends, Prins et al. 2010).

In addition to rhizome expression, we also measured the 'rhizome distances' (RD), i.e. from every rhizome-derived shoot to the center of the crown that it grew from. The average of RD for each plant in a plot was used in the analysis. We normalized the data by taking the log (n+1) value of each phenotype. The transformed data were fitted into a two-part model (Broman 2003), since many individuals with no rhizome expression or intermediate rhizome expression have average rhizome distances of zero. QTLs for rhizomatousness identified in this model can provide a better understanding of their effect (Broman and Sen 2009).

QTL nomenclature used a system that was described in rice (McCouch, Cho et al. 1997), starting with a 'q', followed by an abbreviation of each trait (RZ, RN,RD), then the chromosome number, and finally by a number to differentiate multiple QTLs on a chromosome.

Results

We will discuss three different analyses about rhizomatousness in succession, comparing the genomic regions identified with those found in a previously described F2 population (Paterson, Schertz et al. 1995), and with vegetative branching patterns.

Genomic regions conferring rhizomatousness

We first conduct an analysis to discover genomic regions that are statistically associated with presence or absence of rhizomatousness (RZ), using a Fisher's exact test with a significance level of 0.05 for each marker. We inferred a genomic region or single marker to be significantly associated with rhizomatousness if it met two criteria: 1) a significant association when using a stringent threshold (Benjamini and Hochberg Step-up Method); 2) adjacent markers are significant at the threshold of 0.05. A total of 11 genomic regions associated with rhizomatousness are detected in three different years and four regions are consistently significant in at least two environments (Table 4.1, Figure 4.2). *S. propinquum* alleles increase the degree of

rhizomatousness for all except two regions (qRZ4.1 and qRZ4.2) on chromosome 4 in 2011. This method is easy to implement, and the significant genomic regions can be located in the genome with the aid of the genetic map (Kong, Jin et al. 2013), providing useful information for further comparative study.

The number of rhizomes and the degree of their growth are substantially influenced by environmental factors. In a previous study, the three detected QTLs for LAR (log (n+1) of the number of rhizomes that produced above-ground shoots) only explained 21.8% of phenotypic variation (Paterson, Schertz et al. 1995). In the present experiment, environment remains an essential factor influencing rhizomatousness, shown by the different rhizome expressions in different years (Figure 4.1) and the resulting different sets of genomic regions controlling rhizomatousness (Table 4.1). However, the reoccurrence of genomic regions for rhizomatousness in multiple environments validates some previously reported QTLs for rhizomatousness and facilitates the comparison of their relationships with vegetative branching.

QTL mapping of rhizome number and distances

The second method for evaluating rhizomatousness is to conduct QTL mapping for the number of rhizomes (RN). A total of 5 different QTLs for RN are discovered in three years, and *S. propinquum* contributes all alleles increasing rhizomatousness (Table 4.2 and Figure 4.2). QTLs detected can only account for relatively small amounts of phenotypic variance, 24.44% in 2009, 16.08% in 2010, and 15.03% in 2011, respectively. This is in accordance with our previous study that QTLs detected only explained 21.8% of phenotypic variance (Paterson, Schertz et al. 1995). Four out of five QTLs (qRN1.1, qRN1.2, qRN7.1, qRN8.1) discovered in this method overlap the consensus genomic regions for rhizomatousness in the first method.

Providing more details about the QTL peak and better estimates of QTL effect, the method used for QTL mapping here is more informative than the Fisher's exact test.

The third analysis is to evaluate rhizome distances (RD), which can be fitted to a two-part model (Broman 2003). This essentially involves initial analysis of binary data (presence versus absence of rhizomes), and then subsequent re-analysis of the subset of individuals that have rhizomes. These two procedures can be combined into a two-part model. Thus, the QTLs identified in Table 4.3 have three LOD values. The LOD value, lod.p.mu, indicates the combined effect of both presence/absence and distances of rhizome-derived shoots. The LOD value, lod.p. indicates the presence/absence of rhizomes, expected and found to be similar to that in Table 4.2, except for the QTL on chromosome 1 in 2011. This position on chromosome 1 might be confounded by large segregation distortion near the centromeric region on this chromosome. After we refine this QTL using multiple QTL methods, its position shifts and is similar with that in Table 4.2 (data not shown). The LOD value lod. mu indicates significant QTL for rhizome distances, with only two found. The first one is on chromosome 1, at the interval *CA090231-Xcup06*, affecting both occurrence of rhizome derived shoots and their average distances from the crown. However, significant evidence is only found in one year (2009). Another QTL affects only rhizome distances, located at the interval *Xcup73-Xcup22* on chromosome 1 in 2011. The two-part model has a higher significance threshold than conventional interval mapping and lowers the power of QTL detection. Another drawback of this study is that the sample size of plants with rhizomes is quite small to detect QTL using traditional interval mapping.

Rhizomatousness vs. vegetative branching

In this study, a total of 7 regions for RZ overlap with vegetative branching of different levels (Table 4.1). Three regions, on chromosomes 1, 4, and 7, overlap with tillering QTLs (TL).

Since tillers and rhizomes both develop from axillary buds at basal nodes, this correspondence supports our hypothesis that tillers (stems) and rhizomes may be influenced by overlapping sets of genes and share some biochemical pathways during early developmental stages. Other rhizomatousness regions show correspondence either with a QTL influencing the number of axillary branches (AX) or with the potential for producing secondary or tertiary branches (SR and TR), exemplified by qRZ3.1 and qRZ8.1. Both rhizomes and axillary branches grow basipetally, suggesting that this genomic region might be important in controlling the orientation of the outgrowth of axillary buds.

Comparison to prior studies

Comparing the results from this study with an F2 population from the same cross (Paterson, Schertz et al. 1995) provide additional evidence toward QTL validation (Table 4.1 and Figure 4.2). All three QTLs conferring LAR (Log (n+1) of the number of rhizomes producing rhizomes) in the F2 correspond to two significant genomic regions (qRZ1.1, qRZ1.2) and two QTL for RN (qRN1.1 and qRN1.2) on chromosome 1 in the present study. The QTLs, qRZ1.2 and qRN1.2, on chromosome 1 overlap with two previous LAR QTLs that also overlap with two QTLs for underground rhizomatousness (LSR) in the F2. One of these overlapping QTL for LAR, at the interval *pSB300a-pSB088* in the F2, accounts for the largest phenotypic variation found (Paterson, Schertz et al. 1995; Washburn, Murray et al. 2013), and a more recent study addresses the same regions containing QTLs for rhizome-derived shoots, overwintering and rhizome distances (Washburn, Murray et al. 2013). The facts that both regions (qRZ1.1 and qRZ1.2) are significant under a more stringent statistical threshold in this study, and the corresponding QTLs previously discovered explain a large portion of phenotypic variation,

increase the probability to discover genes responsible for rhizomatousness in these two regions on chromosome 1.

We identified two consensus genomic regions that confer above-ground rhizomatousness in the RILs but were not found in the F2 population, perhaps due to multi-year phenotypic evaluation of the RILs. The consensus genomic regions (Table 4.1, qRZ7.1) and the QTL for RN (qRN7.1) on chromosome 7 were not previously associated with rhizomatousness but overlap with another previously detected trait, regrowth (RG). The prior study showed a clear correlation between rhizomatousness and regrowth, and it is very likely that these QTLs were simply not evident in the single-year prior study based on single-plant measurements.

The previous study detected eight QTLs conferring LSR, four of which can be re-identified by RZ in one or more years in the present study (Table 4.1). The correspondence of these genomic regions substantiates the previous findings, and supports prior evidence of inter-relationship between the rhizomatousness traits. This is particularly true for the relationship between LAR and LSR, since the expression of LAR relies on the expression of LSR.

Discussion

The RIL population derived from the annual parent *S. bicolor* and the perennial parent *S. propinquum*, offers excellent opportunities to study rhizomatousness, a trait highly associated with perenniality. As rhizomatousness is largely influenced by environment, we explored three different methodologies to evaluate the genetic determinants of presence and degree of rhizomatousness. Multi-year testing and re-identification of rhizomatousness validate the positions and effect of previously detected QTLs for rhizomatousness, and the consensus regions discovered may accelerate the identification of genes and characterization of biochemical pathways for rhizomatousness. Comparison of rhizomatousness and vegetative branching is

unprecedented to our knowledge, providing a solid basis for further understanding both the general and differential genetic control of these two traits.

The reproducible nature of the RIL population enables evaluation of rhizomatousness in multiple environments, and thus permits identification of consensus regions that repeatedly show statistically-significant evidence, validating the genomic regions associated with this environmentally-sensitive trait and improving the quality of comparative mapping. In this study, we substantiate our previous result by re-identifying two genomic regions controlling above-ground rhizome shoots on chromosome 1, and showing their correspondence with QTL conferring the same trait in the F2 generation (Paterson, Schertz et al. 1995). The QTL, qRZ1.2, on chromosome 1 in this study has found concordance to not only LAR and LSR QTL in our previous study, but also in other studies. A recent study has also identified two new QTLs for overwintering that overlap with previously detected rhizome QTL in this region, and fine mapping significantly narrowed the area for rhizomatousness to 14.5Mb (Washburn, Murray et al. 2013). Moreover, comparative study of rhizomatousness between sorghum and rice has revealed correspondence of QTLs in several genomic regions (Hu, Tao et al. 2003). *Rhz2* in rice is associated with LAR and LSR QTLs detected in sorghum (Paterson, Schertz et al. 1995), and is also related to qRZ1.2. This non-random occurrence of rhizomatousness on chromosome 1 (qRZ1.2 in Table 4.1) tested both within and between species suggests that genes controlling rhizomatousness and regrowth will be very likely to be identified in this region.

The discovery that seven regions conferring rhizomatousness correspond with branching QTLs, supports the intriguing hypothesis that above-ground vegetative branching and below-ground rhizome growth are related to each other. Rhizomes and stems are developmentally related, with many of the same genes expressed in each of these tissues (Jang, Kamps et al. 2006).

QTLS for rhizomatousness also correspond with those for higher-orders of vegetative branching. This correspondence could be related to genes controlling initiation of all axillary buds, or to orientation of axillary buds, as exemplified by the cases on chromosomes 3 and 8 where the rhizomatousness region overlaps with a QTL specific to axillary branches but not tillers. However, the buds of tillers and rhizomes exhibit a clear positional gradient and undergo divergent development. Tillers re-allocate most resources to inflorescences and are subject to senescence, while rhizomes store carbohydrates and balance their nutrition for overwintering and perennial growth. The QTL, qRZ1.1, chromosome 1 (Table 4.1) exemplifies genomic regions conferring only rhizomatousness, not vegetative branching. The genomic regions identified here may be a fundamental basis for further studying tissue-specific genes for both vegetative branching and rhizomatousness.

Dissecting the genetic components that are important in biochemical pathways and regulatory signals for rhizomatousness may facilitate a wide range of applications. Breeding for perenniality has been recently called for to meet new challenges such as increasing demand for cellulosic biofuel grown on low input land, adapting to changing environmental conditions and preserving ecological capital (especially mitigating soil erosion). Better understanding rhizomatousness may enhance the productivity and perenniality of many forage grasses and many biofuel-dedicated plants. On the other hand, understanding the regulation of rhizomatousness may also make it possible to identify plant growth regulators that precisely target weed control, i.e. to address weeds such as johnsongrass in crops to which they are closely-related, such as sorghum. An ongoing goal is to further investigate the genetics of rhizomatousness in other sorghum populations with more extensive rhizome expression, and in climates in which they overwinter so that regrowth can be measured. Increasing knowledge and

invigorating efforts in discovering rhizome-specific genes and their functions will shed new light upon understanding the formation of rhizomes, developing genetic tools and profiles that will be useful for either enhancing or suppressing rhizomes.

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Table 4.1: Genomic regions conferring rhizomatousness in 2009, 2010 and 2011.

Name	Interval	Genetic location	Physical position[a]		Year						Overlap branching QTL	Overlap F2
					2009	Effect [b]	2010	Effect	2011	Effect		
qRZ1.1	Xcup27-Xcup08 (1)	0-28.1	1.8	7.9	**	-0.18	**	-0.15				LAR, LSR
qRZ1.2	Xcup22-Xcup44 (1)	45.8-70.4	28.2	66.9	**	-0.12	*	-0.11	**	-0.10	TL, AX, VG	LAR, LSR, RD
qRZ3.1	CA187839a-CA152937a (3)	40-50.2	3.5	7.2			*	-0.10			AX, TR, IM, VG	
qRZ3.2	CA157669a-TC48056a (3)	63-66.1	11.9	51.2			*	-0.09			SR	
qRZ3.3	CA146183a-CA187645a (3)	75.1-84.3	52.3	55.6			*	-0.11				
qRZ4.1	CA077825-Xisep0203 (4)	30.2-42.5	5.1	10.0					**	0.02		LSR, RG
qRZ4.2	Xcup71-Xtxp265 (4)	62-77.2	58.0	64.9					*	0.07	TL	
qRZ5.1	CA196148b (5)	74.6	-	-					**	-0.07		
qRZ7.1	Xcup33-Xisep0829 (7)	24.3-47.8	8.6	59.4	**	-0.09			**	-0.11	TL, SR, VEG	RG
qRZ8.1	Xtxp273-Xcup27 (8)	0-7.5	0	3.0	**	-0.11	**	-0.11			AX, SR, MA, IM	
qRZ9.1	CA142735a-Xgap32 (9)	26.5-44.1	31.7	55.1					**	-0.06	TR	LSR

[a] Based on flanking DNA marker locations in the published genome sequence (Paterson, Bowers et al. 2009)

[b] Effect are calculated as *S. bicolor*, BTx623

Table 4.2: Genomic regions conferring number of rhizomes in 2009, 2010 and 2011.

Year	QTL	QTL name	LOD	Interval	Position	Additive effect[a]	% Phenotypic variance explained	Start [b]	End
2009	Full model		9.43				24.44		
	1	qRN1.1	5.46	CA090231b-Xcup06	19	-0.35	13.3	2.9	7.4
	7	qRN7.1	2.51	CA148166b-Xcup70	30	-0.14	2.85	0.9	57.4
	8	qRN8.1	2.15	Xtxp273-Xtxp047	7.5	-0.18	4.4	0.2	3.0
2010	Full model		6.05				16.08		
	1	qRN1.1	3.07	CA090231b-Xcup06	21	-0.19	3.56	2.9	7.4
	3	qRN3.1	2.48	TC58701b-TC69429	80	-0.21	4.63	7.8	End
	8	qRN8.1	2.53	Xtxp273-Xtxp047	1	-0.19	4.47	0.2	3.0
2011	Full model		4.99				15.03		
	1	qRN1.2	3.20	TC59518b-CA078376a	55	-0.15	8.29	28.2	60.8
	7	qRN7.1	2.53	CA193820b-CA141383b	46	-0.11	6.50	8.4	58.2

[a] Additive effect, calculated as *S. bicolor* BTx623

[b] Based on flanking DNA marker locations in the published genome sequence (Paterson, Bowers et al. 2009)

Table 4.3: Genomic regions conferring rhizome distances.

Year	Chr	Interval	Position	Effect	lod.p.mu[a]	lod.p[b]	lod.mu[c]	Start[d]	End
2009	1	CA090231b-Xcup06	20	0.35	6.1	3.6	2.4	2.9	7.3
	7	CA148166b-Xcup70	30	0.15	3.2	3.2	0.1	0.9	57.4
	8	Xtxp273-Xtxp047	6	0.18	2.7	1.2	1.5	0.1	3.0
2010	1	CA090231b-Xcup06	21	0.19	3.1	2.9	0.2	2.9	7.3
	3	TC58701b-TC69429	80	0.20	2.6	2.1	0.7	7.8	end
	8	Xtxp273-Xtxp047	0	0.19	2.7	2.3	0.4	0.1	3.0
2011	1	Xcup73- Xcup22	39	0.17	3.9	0.1	3.8	15.2	61.6
	7	CA193820b-CA141383b	46	0.10	3.4	3.3	0.1	8.4	59.0

[a] Lod.p.mu reflects both the occurrence of rhizomatousness, and their distances

[b] Lod.p: reflects the occurrence of rhizomatousness

[c] Lod.mu: reflects the distances of rhizomes from the source crown

[d] Based on flanking DNA marker locations in the published genome sequence (Paterson, Bowers et al. 2009)

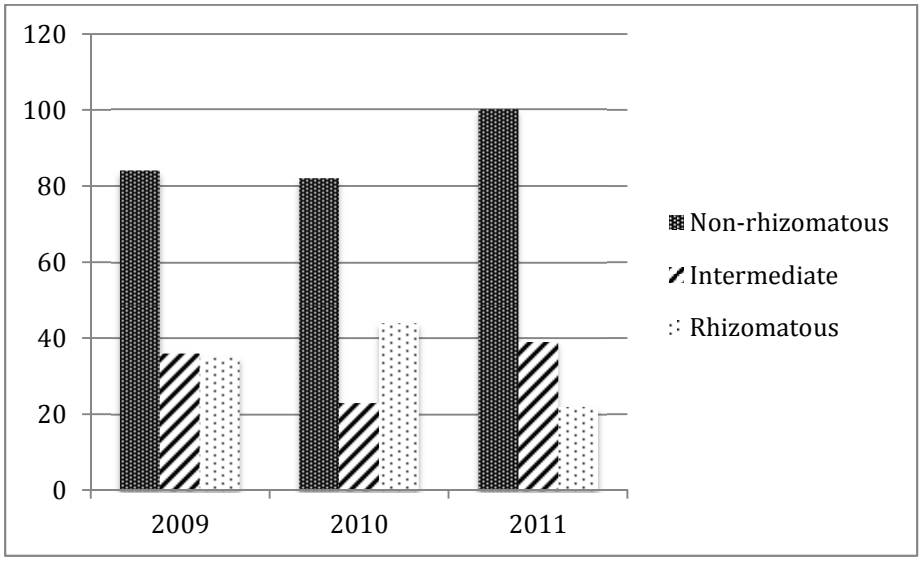
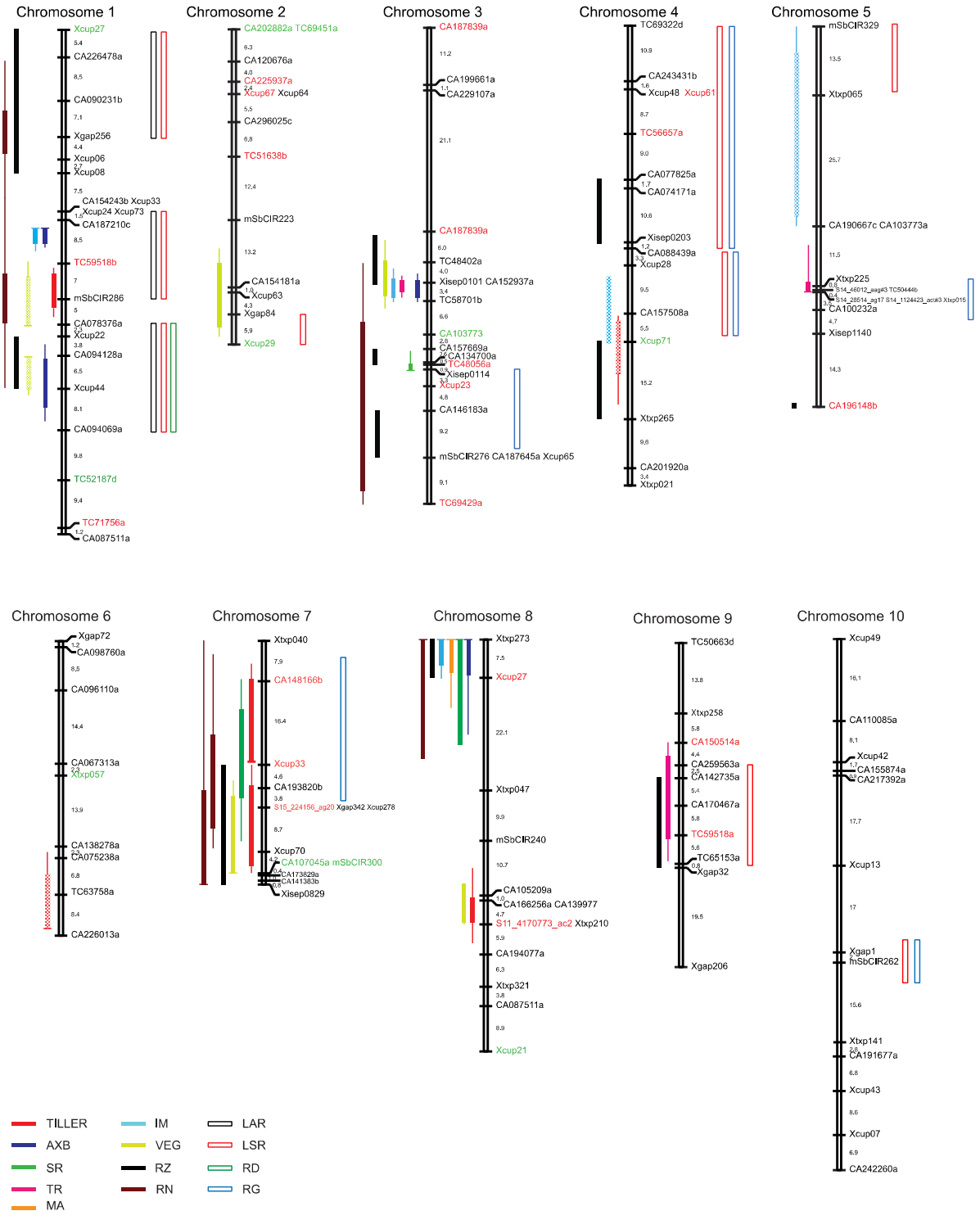


Figure 4.1: Presence of rhizomes in three years.

Figure 4.2: Mapping of rhizomatousness QTLs in the *S. bicolor* × *S. propinquum* RILs (left), and comparison to vegetative branching (left) and a previous F2 population (right). QTLs are shown with 1-lod (solid or dotted) and 2-lod (whiskers) intervals. Significant genomic regions for rhizomatousness (RZ) are only shown with solid boxes. For vegetative branching QTLs, solid boxes indicated that the QTL is significant after 1000 permutation tests, and dotted boxes are 'putative' QTLs only significant at a LOD score of 2. Markers whose physical locations are unable to be located to their corresponding chromosomes are in red; markers whose genetic orders disagree with their physical locations are in green.


Chromosome 1
Xcup27
CA226478a
CA090231b
Xgap256
Xcup06
Xcup08
CA154243b Xcup33
Xcup24 Xcup73
CA187210c
TC59518b
mSbCIR286
CA078376a
Xcup22
CA094128a
Xcup44
CA094069a
TC52187d
TC71756a
CA087511a
Chromosome 2
CA202882a TC69451a
CA120676a
CA225937a
Xcup67 Xcup64
CA296025c
TC51638b
mSbCIR223
CA154181a
Xcup63
Xgap84
Xcup29
Chromosome 3
CA187839a
CA199661a
CA229107a
CA187839a
TC48402a
Xisep0101 CA152937a
TC58701b
CA103773
CA157669a
CA134700a
TC48056a
Xisep0114
Xcup23
CA146183a
mSbCIR276 CA187645a Xcup65
TC69429a
Chromosome 4
TC69322d
CA243431b
Xcup48 Xcup61
TC56657a
CA077825a
CA074171a
Xisep0203
CA088439a
Xcup28
CA157508a
Xcup71
Xtxp265
CA201920a
Xtxp021
Chromosome 5
mSbCIR329
Xtxp065
CA190667c CA103773a
Xtxp225
CA100232a
Xisep1140
CA196148b
Chromosome 6
Xgap72
CA098760a
CA096110a
CA067313a
Xtxp057
CA138278a
CA075238a
TC63758a
CA226013a
Chromosome 7
Xtxp040
CA148166b
Xcup33
CA193820b
S15_224156_ag20 Xgap342 Xcup278
Xcup70
CA107045a mSbCIR300
CA173829a
CA141383b
Xisep0829
Chromosome 8
Xtxp273
Xcup27
Xtxp047
mSbCIR240
CA105209a
CA166256a CA139977
S11_4170773_ac2 Xtxp210
CA194077a
Xtxp321
CA087511a
Xcup21
Chromosome 9
TC50663d
Xtxp258
CA150514a
CA259563a
CA142735a
CA170467a
TC59518a
TC65153a
Xgap32
Xgap206
Chromosome 10
Xcup49
CA110085a
Xcup42
CA155874a
CA217392a
Xcup13
Xgap1
mSbCIR262
Xtxp141
CA191677a
Xcup43
Xcup07
CA242260a
TILLER
AXB
SR
TR
MA
IM
VEG
RZ
RN
LAR
LSR
RD
RG

CHAPTER 5

CONCLUSION

Plant architecture is the three dimensional organization of a plant body, determined by the sizes and shapes of plant organs, and patterns of above-ground vegetative branching and underground growth by roots and rhizomes (subterranean stems). Plant architecture decides the dispositions of vegetative organs that capture light, and the synchrony of inflorescence and seed development that are important factors for grain production. Sorghum is an excellent model in which to study plant architecture, thanks to its rich morphological diversity occurring during divergent evolution both in the wild and under domestication for a variety of purposes (food, forage, sugar, biomass, and special uses such as 'broomcorn'). Abundant genetic tools and resources (Paterson, Bowers et al. 2009) have made it possible to identify the genomic regions or genes that underlie variations in plant architecture, which will shed new light upon a variety of applications ranging from plant growth regulation to breeding for perenniality.

The RIL population derived from two morphologically distinct parents, *S. bicolor* and *S. propinquum*, displays large phenotypic variations and offers new opportunities for discovering genes, biochemical pathways and plant growth regulation. Advanced in a temperate climate (Lubbock, TX), the RIL population improves the ability to discover QTLs by eliminating confounding factors that are correlated with short-day flowering from *S. propinquum*. The homozygous nature of the RIL population enables evaluation in multiple environments, and thus makes the analysis of genotype and environmental effects of a trait feasible and the results of

QTL mapping more reliable. This is particularly important for relatively ‘plastic’ traits such as the components of plant architecture that we have investigated here.

Our phenotypic system for vegetative branching is novel to our knowledge, distinguishing genomic regions that have a general influence on all vegetative branching from those with distinct control of specific levels of branching. Our study showed large correspondence of branching QTLs with previously reported QTLs controlling tillering. Specifically, we validated two previously discovered tillering QTLs (Paterson, Schertz et al. 1995) and detected two that had not been previously found in an F2 population with the same parentage as ours, but which can be related to QTLs reported in other publications (Hart, Schertz et al. 2001; Shiringani, Frisch et al. 2010). Other QTLs for vegetative branching traits addressed here are new and important in determining the final architecture of a plant. Genes in these QTL regions can be further identified to design an ideal genotype for different human uses.

A total of five genomic regions underlying rhizomatousness show correspondence with either the same trait, above-ground shoots derived from rhizomes, or other rhizomatous related traits, reported in a previous study. Since rhizomatousness is greatly influenced by environment, the repetition of findings in multiple environments is important for QTL validation. Further, genomic regions influencing rhizomatousness also overlap with different levels of vegetative branching QTLs, suggesting that these two traits might share overlapping sets of genes at early developmental stages.

Identifying genomic regions influencing vegetative branching and rhizomatousness facilitates a wide range of applications. The QTL information can be directly used for marker-assisted selection in sorghum breeding programs. Isolating genes for vegetative branching and rhizomatousness can benefit plant growth regulation: up-regulation of both branching and

rhizomatousness may enhance the productivity and perenniality of many forage and biomass dedicated crops for food and biofuel, while down-regulation may improve grain production and control many noxious weeds. Increasing knowledge of genetic determinants of plant architecture that are crucial to food and biofuel productivity may shed new light upon developing new genotypes optimized for sustainable food and cellulosic biomass production.

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Paterson, A., H., J. E. Bowers, et al. (2009). "The Sorghum bicolor genome and the diversification of grasses " Nature **457**: 551-556.

Paterson, A. H., K. F. Schertz, et al. (1995). "The Weediness of Wild Plants - Molecular Analysis of Genes Influencing Dispersal and Persistence of Johnsongrass, Sorghum-Halepense (L) Pers." Proceedings of the National Academy of Sciences of the United States of America **92**(13): 6127-6131.

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