INTEGRATED ANALYSIS OF PHENOLOGY, TRAITS, AND QTL IN THE DROUGHT TOLERANT SORGHUM GENOTYPES BTx642 AND RTx7000

A DISSERTATION

by

BROCK D. WEERS

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

August 2011

Major Subject: Molecular & Environmental Plant Sciences

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Approved by:

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ABSTRACT

Integrated Analysis of Phenology, Traits, and QTL in the Drought Tolerant Sorghum Genotypes BTx642 and RTx7000. (August 2011)

Brock D. Weers, B.S., Iowa State University

Chair of Advisory Committee: Dr. John Mullet

The growth and development of two sorghum drought tolerant genotypes BTx642 (post-flowering drought tolerant, "stay green") and RTx7000 (pre-flowering drought tolerant) were characterized and compared. Differences in phenology and the growth and development of leaves and stalks were identified that could contribute to variation in shoot biomass, grain yield and response to water deficit. An F_{12} recombinant inbred line (RIL) population derived from the two parents was genotyped using the Illumina Genome Analyzer II platform and the information used to generate a genetic map useful for analysis of quantitative trait loci (QTL). Seventy-two different traits were measured in the RIL population at anthesis and at grain maturity. Plants were grown in well-watered environments in greenhouse conditions and in field conditions near College Station, TX in 2008-2010. QTL mapping was used to analyze the genetic basis of trait variation in the population and to detect associations between traits.

A total of 477 QTL were identified that in combination modulate leaf size (length, width, and area), shoot biomass accumulation (shoot, stalk, stem, leaf, and leaf sheath), panicle weight, root size and architecture (length, surface area, and volume,

number of tips, forks and nodal roots, and root biomass), stalk and stem length, and flowering time. Six flowering time QTL were identified and variation in time to anthesis affected the expression of several other traits including leaf size and biomass accumulation. However, QTL infrequently had an impact on traits associated with different organs. The specificity observed is consistent with independent genetic control of traits associated with leaves, stems and roots. Nine QTL that modulated shoot biomass accumulation were detected that were not affected by flowering time. Of these, four shoot biomass QTL co-localized with leaf size traits. Eight QTL for panicle biomass were detected with two coincident with QTL for upper leaf size. A QTL for leaf width at anthesis was found to co-localize with a stay green locus.

NOMENCLATURE

ABA	Abscisic acid
BAC	Bacterial artificial chromosome
cM	Centimorgan
DAE	Days after emergence
DTF	Days to flower
Kbp	Kilo base pair
LG	Linkage group
LOD	Log of odds
Mbp	Mega base pair
QTL	Quantitative trait loci
RIL	Recombinant inbred line

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CHAPTER I

INTRODUCTION

Sorghum Origins

Drought is defined as a prolonged or chronic shortage of rainfall (Mish and Morse, 2005). However, water limitation of any duration causes loss of crop yield by inhibiting growth. In the United States, with its population of 307 million people (U.S. Census Bureau, 2009), drought causes an estimated $6-8 billion in crop damage annually (FEMA, 1995). In other more populated parts of the world drought is even more commonplace. In heavily populated countries such as China and India, ranking number one and number two in world population at 1.34 billion people and 1.16 billion (U.S. Census Bureau, 2009) respectively, drought can have massive economic impact and disrupt food supply. To this end, it is of key importance to develop drought tolerant economically important crop species that employ strategies to maintain grain yield under water-limiting conditions. One such crop that meets this criterion is sorghum (*Sorghum bicolor* [L.] Moench).

Sorghum is a member of the Poaceae tribe, which includes rice, maize, barley, oat, rye, millet, and wheat. This species diverged from maize ~10-20 million years ago and rice ~50 million years ago (Doebley et al., 1990). However, gene order along homeologous chromosomes remains highly conserved between rice and sorghum, allowing for comparative genomic mapping (Bennetzen, 2000). *Sorghum bicolor*

This dissertation follows the style of Crop Science.

(2n=10) has an ~730 Mbp genome (Patterson et al., 2009). This C4 plant is the fifth most important cereal worldwide for human use and the 2nd most important crop grown for animal feed (Doggett, 1988). Additionally, a high density genetic map containing 2926 loci (Menz et al., 2002) derived from a BTx623/IS3620C RIL population, a BAC-based physical map linked to this genetic map (Klein et al., 2000), a cytogenetic map of chromosomes (Islam-Faridi et al., 2002; Kim et al., 2005), 550,000 methyl-filtered sequences (Bedell et al., 2005), 117,682 ESTs (Pratt et al., 2005), and a complete sequence of the genome (Patterson et al., 2009) are all available to accelerate research on sorghum.

Sorghum has been divided into five main races that are distributed in different regions of Africa: the guinea, bicolor, durra, caudatum, and kafir races. This distribution reflects the climatic zones of Africa, which radiate outward from the continent's center. The climate of central Africa is one of a tropical rainforest, receiving 180+ cm of rain each year (Stock, 1995). This is where the guinea race predominates (Smith and Fredericksen, 2000). The next climatic zone outward from the tropical rainforest is the tropical savannah, receiving 50-100 cm of rainfall per year (Stock, 1995). Caudatum, bicolor, and to a lesser extent, kafir, are widely distributed throughout this climatic zone (Smith and Fredericksen, 2000). The tropical savannah transitions to the steppe climatic zone that receives only 25-50 cm of rain per year (Stock, 1995). This region is predominated by durra sorghums in the north and kafir sorghums in the south (Smith and Frederiksen, 2000). Lastly, northern Africa and the southern extremities of Africa are

desert, receiving less than 25 cm of rainfall per year (Stock, 1995). No sorghum races predominate in this region.

Drought Escape, Dehydration Avoidance, and Dehydration Tolerance

Among crop plants, sorghum is regarded as a very drought tolerant species. Three chief mechanisms enable higher plants to deal with drought conditions (Levitt, 1980): 1) Drought Escape: the plant completes its life cycle during favorable conditions, thus "escaping" dry environments. 2) Dehydration Avoidance: the plant maintains cell turgor via increased water uptake by expanding the root system or decreasing transpirational water loss. 3) Dehydration Tolerance: metabolism is maintained at low cell turgor both by increasing compatible solute concentration (which decreases osmotic potential) and by increasing the antioxidant capacity of the plant.

One method by which sorghum mediates drought escape is through modulation of flowering time. Sorghum is a short day plant that can also modulate flowering time based on temperature input (Quinby et al., 1973). Therefore, flowering in photoperiod sensitive sorghum genotypes is delayed when day lengths are above a critical photoperiod threshold (Blum, 2004; Ellis et al., 1997). Photoperiod sensitivity varies among sorghum genotypes and this property is used to alter time to flowering in different grain sorghums in order to complete the plant's life cycle before commencement of the dry season.

A plant's total biomass is linearly correlated to total transpiration and water use (Hammer et al., 1997). Therefore, in water-limited environments, modulation of plant transpiration and water use is an important element in adaptation. One way this is done

is by altering leaf area, demonstrated in a study in which the bulk of transpiration differences across diverse sorghum genotypes were due to differences in leaf area (Mortlock and Hammer, 1999). Additional mechanisms that help plants avoid dehydration include leaf wilting, folding, erectness, and rolling (Wilson et al., 1980; Ludlow and Bjorkman, 1984). Through these leaf movements, solar radiation on the leaf surface is reduced, helping to keep the leaves cooler, thus reducing the loss of water (O'Toole et al., 1979). Sorghum is also characteristically known to produce an epicuticular wax on its leaves and leaf sheaths that reflect light, thus reducing leaf temperature and water loss (Johnson et al., 1983). Dehydration avoidance is also correlated to root properties, such as root depth and density, shown by Wright and Smith (1983) in which a drought tolerant sorghum genotype had deeper roots with higher density than a drought susceptible genotype when grown under water-limiting conditions. The deeper rooting likely contributed to the plant's ability to remain hydrated and continue to accumulate biomass normally, hence avoiding dehydration.

Dehydration tolerance is conferred both through lowering water potential by decreasing osmotic potential and by increasing antioxidant capacity. In sorghum, sugars and small ions such as potassium and chloride are the main solutes contributing to osmotic potential (Premachandra et al., 1995). Wright and Smith (1983) and Morgan (1984) found that increased solute concentration can decrease the rate of leaf senescence through increasing dehydration tolerance. This study was corroborated by Basnayake et al. (1993) in that maintenance of turgor through increased solute concentration was found to be involved in keeping sorghum alive for ten days of terminal drought stress.

Additionally, other studies have shown that increasing solute concentration allows the stomata to remain partially open under increasing drought stress (Ludlow et al., 1985; Ludlow, 1987). Several other solutes have been found to increase in concentration in the cell in response to gene induction under water-limiting conditions, which suggests that these genes may play a role in dehydration tolerance as well. Examples include glycine betaine from induction of betaine aldehyde dehydrogenase (Wood et al., 1996) and accumulation of proline, composing up to 60% of the free amino acid pool. Dehydrins are also among the first proteins observed in the cell in response to drought stress and are known to act as membrane stabilizers under low water potential (Danyluk et al., 1998; Ismail et al., 1999).

Dehydration tolerance is also conferred by production of reactive oxygen species. These molecules have been shown to increase in concentration and contribute to tolerance to abiotic stresses, including drought, dessication, salt, chilling, heatshock, heavy metals, UV, and ozone (Hammond-Kosack and Jones, 1996). Reactive oxygen species are thought to be signaling molecules for abiotic stresses (Desikan, 2001). The plant responds by producing antioxidants, which function in neutralizing excess reactive oxygen species not used in signaling cascades (Scandalios, 2005). Examples of enzymes involved in protection from reactive oxygen species include superoxide dismutase, catalase, and peroxidase. These enzymes use free radical scavengers such as carotenoids and glutathione to neutralize reactive oxygen species (Mundree et al., 2002).

ABA Regulation

Abscisic acid (ABA) is a hormone integrally involved with drought conditions. It is well established that ABA levels rise due to increased ABA synthesis in response to a water deficit that causes loss of cell turgor (Beardsell and Cohen, 1975; Davies and Zhang, 1991; Allan et al., 1994; Millborrow et al., 2001). The plant responds to higher levels of ABA by increasing its root-to-shoot dry weight ratio, inhibiting leaf area development (Sharp et al., 1994), closing stomata (Ward et al., 1995), increasing hydraulic conductance for water movement from roots to shoots (Saab, 1990), and maintaining cell turgor by inducing the synthesis of compatible solutes and protective proteins such as dehydrins, LEA, etc (Bray, 1993). Several QTL for ABA accumulation have been identified in cereal species such as maize (Tuberosa et al., 1998) and barley (Sanguineti et al., 1996). Additionally, genes such as *ABI1* and *ABI2* from *Arabidopsis thaliana* have been cloned and shown to be negative regulators of ABA expression in maize protoplasts (Sheen, 1996).

The 5' UTR of many ABA-responsive genes contains an AT-rich region, an ABA responsive element (c/tACGTggc), and a GC-rich coupling element (Himmelbach et al., 2003). For a maximal ABA-induced response, transcription factors must bind each of these sites (Shen and Ho, 1995). HD-ZIPs such as AtHB5 and AtHB6 bind to the AT-rich region, bZIPs such as ABI5 bind to the ABA responsive element, and AP2s such as ABI4 bind to the coupling element (Himmelbach et al., 2003).

Regulation of ABA induced responses is complex (Shinozaki et al., 2003; Himmelbach et al., 2003). Many studies have been carried out to understand how ABA

modulates transcription. In Arabidopsis, genes known to both be induced by dehydration and increased levels of ABA, such as *NCED3*, *AAO3*, *ABA3*, and *ZEP*, are not induced by glucose, which also has been shown to increase ABA levels (Cheng et al., 2002). In ABA anabolism, *NCED* is a key regulatory step since this gene encodes an enzyme that is the rate limiting step in ABA biosynthesis and high ABA concentrations are correlated with high *NCED* expression (Schwartz et al., 2003). With respect to catabolism, ABA 8'-hydroxylase is proposed to be the main regulatory step (Kushiro et al., 2004; Saito et al., 2004; Xiong and Zhu, 2003). However, overexpression of upstream enzymes such as 1-deoxy-D-xylose-5-phosphate synthase, phytoene synthase, and *ZEP* also cause increased ABA concentrations in the plant (Frey et al., 1999; Lindgren et al., 2003). In addition many of the *NCED* genes are also induced by external application of ABA, suggesting that the hormone may regulate its own accumulation (Xiong et al., 2002; Xiong et al., 2001).

Stay Green

One drought tolerance trait studied in sorghum is the stay green trait, in which plants have delayed post-flowering leaf senescence under water-limiting conditions. Senescence is the final stage in the life of a leaf. This stage is characterized by a decrease in photosynthetic activity, degradation of lipids and proteins, and mobilization of degraded cell components to actively growing parts of the plant, such as the developing seed (Nooden, 1988). Gene expression during this phase of a plant's life cycle has been measured using subtractive hybridization techniques in various species, such as *Brassica oleracea.* Smart (1994) found increased expression of cell wall

degradative enzymes such as pectinesterase and polygalacturonase, chlorophyll degradation enzymes, enzymes of carbohydrate metabolism, protein and amino acid metabolism, nucleic acid metabolism, lipid metabolism, and signal transduction (ethylene receptors, protein kinases). Many of these genes have been isolated and their role in senescence tested directly. For example, in tomato, expression of an antisense copy of ACC oxidase, an enzyme involved in ethylene biosynthesis, delayed fruit ripening and leaf senescence (Picton et al, 1993).

Most of the genes shown to be involved in senescence are regulated by drought, darkness, detachment, ABA, ethylene, and cytokinins (Malik, 1987; Nooden, 1988; Oh et al., 1996). While all of the former are known to induce senescence, high cytokinin levels have been shown to repress senescence (Gan and Amasino, 1996).

One way to significantly delay leaf senescence under water limiting conditions is through the stay green trait. Stay green plants retain greater green leaf area under conditions where senescent genotypes "go brown." Thomas and Howarth (2000) characterized five classes of stay green. Type A pertains to a delay in the onset of leaf senescence, but then normal senescence occurs. Type B initiates senescence at the normal time, but the rate of senescence is slower than normal. Type C plants senesce at the normal time, but chlorophyll degradation does not occur. This latter type of stay green is termed 'cosmetic' stay green in contrast to functional stay-green responses. In Type D plants, rapid tissue death prevents chlorophyll degradation, so this class is cosmetically stay green as well. Finally in Type E, senescence occurs at the normal time and rate; the plant just has an abundance of chlorophyll in its tissues so it is able to

remain green longer than senescent types. The stay green phenotype pertains to post-flowering drought responses seen in species such as sorghum, maize, tomato, and oat. For sorghum, when moisture stress occurs during grain filling, yield is reduced and charcoal rot and lodging ensue (Rosenow and Clark, 1981). In Type A and B stay green plants, however, the plant remains green and continues to fill grain under drought conditions. Even under well-watered conditions yield is not compromised (Borrell et al., 2000). Physiologically, relative to senescent genotypes, stay green genotypes are associated with increased basal stem sugars (Duncan, 1984), increased cytokinins (McBee, 1984), increased nitrogen content (Borrell et al., 2000), and increased transpirational efficiency (Borrell et al., 2000).

Several sources of germplasm for stay green exist. These include four lines: 1) BTx642 (formerly B35), which is derived from IS12555. This line is derived from the durra race, geographically located in Ethiopia. BTx642 is known for pre-flowering drought stress susceptibility, but post-flowering drought tolerance (Borrell et al., 2000). 2) SC56, which is from the caudatum-nigricans race in Sudan. This species is pre-flowering drought-susceptible, post-flowering drought tolerant and tolerant to stalk lodging (Kebede et al., 2001). 3) KS19, which is derived from Combine Kafir and Short Kaura. This line is from Nigeria and is known for a delayed onset of drought stress (Borrell et al., 2000). 4) IS22380 is a line from Sudan and a member of the caudatum race. This line is also known for a delayed onset of drought stress (Mahalakshmi and Bidinger, 2002).

Several QTL for the stay green trait have been localized through analysis of populations involving crosses between senescent and non-senescent sorghum genotypes. Xu et al. (2000), in a RIL population derived from BTx642 and RTx7000 identified four *Stg* QTL. *Stg1* accounted for 20%, *Stg2* 30%, *Stg3* 16%, and *Stg4* 10% of the phenotypic variance. Seven stay green QTL were identified using a RIL population derived from BTx642 and Tx430 (Crasta et al., 1999). *StgA*, *StgD*, and *StgG* accounted for 42% of the phenotypic variability, while *StgB*, *StgI.1*, *StgI.2*, and *StgJ* accounted for 25% of the phenotypic variability. Crasta et al. (1999) found that *Stg2* and *Stg4* from the BTx642/RTx7000 RIL population (Xu et al., 2000) co-localized with QTL of their population. In 2000, Tao and colleagues (2000) identified five stay green QTL (on linkage groups A, B, C, G, and I) from a RIL population derived from QL39 and QL41. Kebede and colleagues (2001) examined a SC56/RTx7000 RIL population and identified three Stg QTL located on linkage groups A, G, and J. Upon correlation of their results with others, they found that the *StgA* and *StgJ* QTL co-localized to *Stg* QTL in both the BTx642/RTx7000 RIL population (Xu et al., 2000) and the BTx642/Tx430 RIL population (Crasta et al., 1999). *StgG* only co-localized with a *Stg* QTL observed in the BTx642/Tx430 population (Crasta et al., 1999).

In sum, sorghum is an invaluable species to use in studying and understanding drought tolerance mechanisms that enable this species to be productive in water-limited environments. In this dissertation traits will be examined concerning how the plant prepares itself for post-anthesis drought under pre-anthesis well-watered environments through the life cycle of the plant. Additionally, since traits will be measured under well-

watered conditions, the scope of the research will be broadened to include shoot biomass accumulation and yield. Traits that may segregate in a recombinant inbred line (RIL) population derived from the parents will first be tested in parental genotypes BTx642 (post-flowering drought tolerant, "stay green") and RTx7000 (post-flowering drought senescent). Those traits with differences between the parents will then be examined in the population through using QTL analyses. Finally, correspondence between component traits and macro traits post-flowering drought tolerance (stay green), shoot biomass, and yield will be examined.

CHAPTER II

GROWTH AND DEVELOPMENT OF BTx642 AND RTx7000

Introduction

BTx642 was identified as a stay green post-flowering drought tolerant sorghum genotype whereas RTx7000 was characterized as a pre-flowering drought tolerant but post-flowering drought susceptible genotype (Duncan et al., 1981; Rosenow et al., 1983). Morphometric traits that can affect plant response to drought stress include leaf and canopy size, stem size, and root length. Stem weight was associated with differences in post-flowering drought stress response in BTx642 possibly because large amounts of carbon can be stored in stems and used for grain filling (Duncan et al., 1981; Borrell et al., 2000a). Canopy size, determined by leaf number and leaf size has been shown to modulate drought responses, with drought tolerant genotypes retaining more green leaf area and having higher yields than senescent genotypes (Borrell et al., 2000). Canopy size, transpiration efficiency and environmental conditions affect the rate of water utilization by plants. Differences in leaf area, surfaces (wax, hairs), and erectness have also been related to variation in water use efficiency and drought tolerance (O'Toole et al., 1979; Wilson et al., 1980; Johnson et al., 1983; Ludlow and Bjorkman, 1984; Mortlock and Hammer, 1999).

Differences in phenology as well as variation in plant morphology can influence plant responses to water deficit. Phenology is the study of cyclic events that occur during plant development and how these events are influenced by climate (environment, seasons). For example, time to flowering and the rate and number of leaves produced

prior to flowering often have a dramatic impact on grain yield in environments subject to drought. Early flowering plants can often 'escape' drought by completing their life cycle prior to water deficit.

In this study, phenological and morphometric traits associated with root and shoot growth were measured to identify potential differences between BTx642 and RTx7000. Differences in traits identified in this study were targeted for further analysis in a RIL population derived from these genotypes, thereby allowing QTL mapping of potentially useful agronomic traits.

Materials and Methods

On 1/23/11 BTx642 and RTx7000 seed were planted in containers containing coarse vermiculite (Sun Gro Horticulture) and germinated under growth chamber conditions of 14 hour days, 31º C during the day and 23º C during the night. Emergence occurred on 1/27/11. At 3 DAE each seedling was transplanted into one 1.5 m tall by 10 cm wide schedule 40 PVC pipe containing Brazos County, TX silty loam soil uniformly mixed with 40 g of slow-release fertilizer, osmocote 14-14-14 (Scotts-Sierra Horticultural Products). Prior to filling the pipes, all soil was sieved through a 6.4 mm screen to remove large pieces of organic and inorganic materials. The plants were grown under greenhouse conditions with an average daily maximum temperature of 34.4º C and an average daily minimum temperature of 23.2º C. All plants were spaced 10 cm apart and grown under well-watered conditions using automatic watering system model 9001DB (DIG Irrigation Products) in conjunction with pressure compensating emitters (DIG Irrigation Products). The system was pressurized at 2.1 bar and the emitters

operated at 3.8 L/hr at any pressure between 1.0-3.8 bar. Using this system, no water was emitted until the system reached 1.0 bar, thus enabling uniform watering across all plants. Excess water was allowed to drain through the bottom of the pipes. Plants were grown under 14 hour days through 16 DAE, followed by progressive day-shortening treatments of 12.5 hour days for two weeks, 11.5 hour days for one week, and 10 hour days for one week. After the 10 hour day treatment, light conditions were returned to 14 hour days for the duration of the experiment. The plants were fertilized every 30 days with 20 g of osmocote 14-14-14 (Scotts-Sierra Horticultural Products).

Plant harvest began at 16 DAE and continued at frequencies of roughly 10 days through 87 DAE. Ten plants were harvested at each time point. Measured traits included individual leaf weight, length, width, and area, leaf sheath weight, stalk and stem lengths and weights, root size parameters, root weight, number and length of nodal roots, and time to flowering. Leaf size parameters were measured with a LI-3100C Leaf Area Meter (LI-COR Biosciences). Root morphometrics were examined through first individually removing washed nodal roots from the root crown and then scanning in an Epson 10000XL dual lens scanner at 600 dpi. Roots of length less than ~1 m were scanned in groups of 2 or more depending on length. Roots greater than ~1 m were scanned individually. The root images were analyzed with WinRhizo V.2008a software (Regent Instruments), which determined total root length, total root surface area, total root volume, total number of root tips and forks, and average root diameter. All tissues were dried at 71º C for three days to determine dry weights.

Significant differences between BTx642 and RTx7000 for a given trait were determined using SigmaPlot V8.02 (Brannan et al., 2001). This program conducted pair-wise independent (two-tailed) *t*-tests and determined significance at $P<0.05$. Trait data were arrayed in SigmaPlot such that for a given trait, one column represented trait values for BTx642 and an adjacent column represented trait values for RTx7000. The *t*-tests were performed by selecting the data for a given trait, selecting "Statistics" in the menu bar, and then selecting "Independent *t*-test." Statistically significant differences between the two genotypes for a given trait were tested under the null hypothesis that the means were equal ($H_0 = 0$) and the alternate hypothesis that the means were not equal ($H_1 \neq 0$). The null hypothesis was rejected in favor of the alternate hypothesis any time P was equal to or less than 0.05 (95% confidence level).

Results

Flowering Time

The development of BTx642 and RTx7000 was compared in plants grown in pipes under greenhouse conditions in shortening days through an extended flowering-inducible phase (~16-46 DAE). Through plant dissection in the course of harvesting stems and leaf sheaths, RTx7000 was determined to be induced to flower at ~36 DAE, and BTx642 at ~60 DAE. RTx7000 reached anthesis at ~62 DAE and BTx642 at ~85 DAE. The time from floral initiation to anthesis (GS2) required ~25-26 days for both genotypes.

Shoot and Leaf Traits

Shoot (stem, leaves, leaf sheaths, and panicles when present) fresh and dry weights were measured at 16 DAE and approximately every 10 days thereafter until anthesis (Fig. 2.1, 2.2). Total shoot biomass of the two genotypes, measured either as fresh weight or dry weight, increased steadily and approximately in parallel during development until 57-67 DAE. Thereafter, the shoot biomass of BTx642 exceeded RTx7000.

Leaf number increased steadily and in parallel for both genotypes until 57 DAE when both plants had a total of 17 leaves, of which 13 were fully expanded in BTx642 and all 17 in RTx7000 (Fig. 2.3). RTx7000 produced no additional leaves after this time point. BTx642 continued to produce leaves at approximately the same rate until 67 DAE reaching a total of 21 leaves, and by 87 DAE all of its leaves were fully expanded. Leaves one through five began to senesce by 36 DAE and by 67 DAE the first five leaves had turned yellow in both genotypes. The rate of leaf senescence decreased from 67 DAE to 87 DAE with only one to two additional leaves showing signs of leaf senescence during this period.

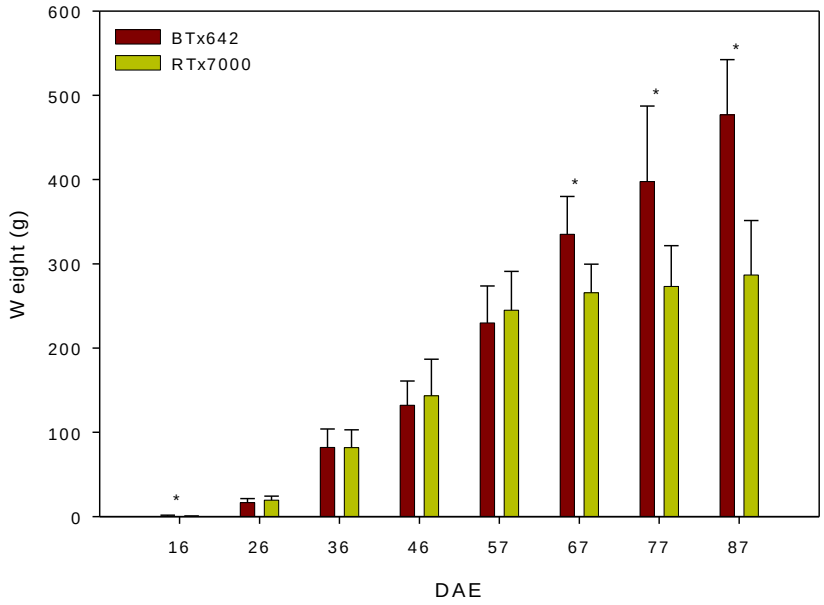


Figure 2.1. BTx642 and RTx7000 shoot total fresh weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

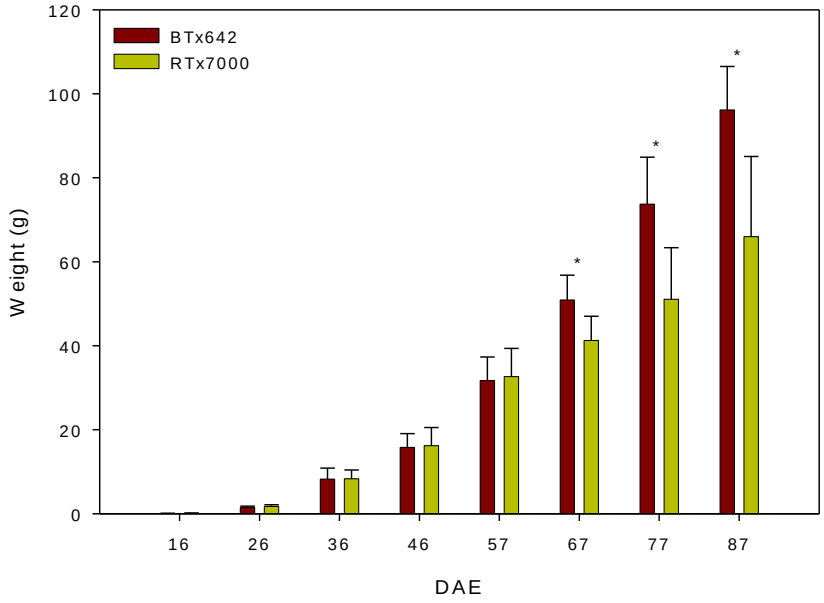


Figure 2.2. BTx642 and RTx7000 shoot total dry weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

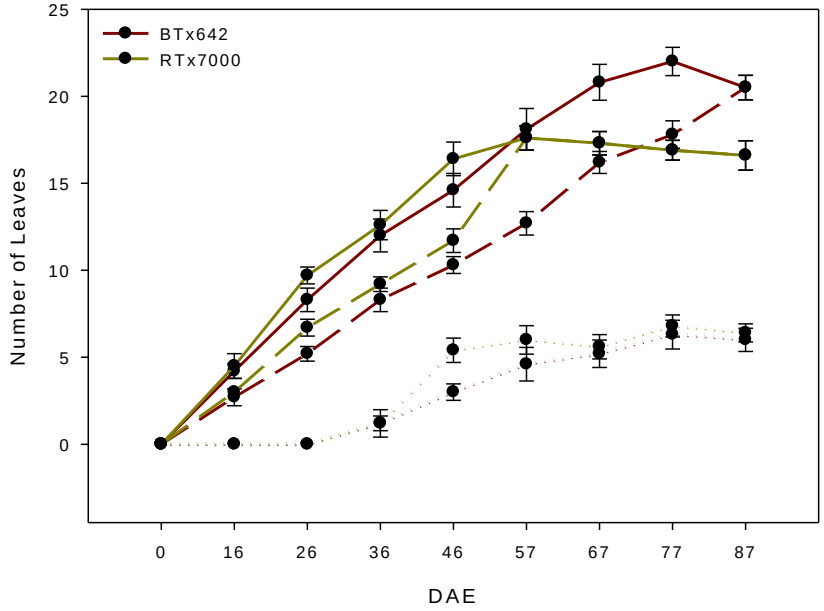


Figure 2.3. BTx642 and RTx7000 total number of leaves (solid lines), fully expanded leaves (dashed lines), and senesced leaves (dotted lines) time course. Data are shown as means ± standard deviation.

Total leaf fresh weight and dry weight of BTx642 and RTx7000 leaves were similar until 57 DAE (Fig. 2.4, 2.5). Total leaf weight increased more rapidly in BTx642 than RTx7000 from 57 DAE to 77 DAE. At that point total leaf fresh weight decreased slightly in BTx642, but both total leaf fresh and dry weight remained well above RTx7000's total leaf weight. The fresh and dry weight of individual leaves of BTx642 and RTx7000 varied significantly and in a way that helped explain differences in total leaf weight (Fig. 2.6, 2.7). Most of the first 10 leaves had more fresh and dry weight in BTx642 than RTx7000; however, RTx7000 consistently had one to two more fully expanded leaves than BTx642 during the first 46 DAE, so total leaf weights were

similar. Then at 57 DAE and especially at 67 DAE, BTx642 had several additional fully expanded leaves that out-weighed RTx7000 leaves. Leaves 14-17 weighed significantly more in BTx642 than RTx7000, contributing to the larger difference in weight seen in later stages of growth. BTx642 produced about 3 more leaves than RTx7000, with the BTx642 flag leaf weighing approximately three times more than the RTx7000 flag leaf.

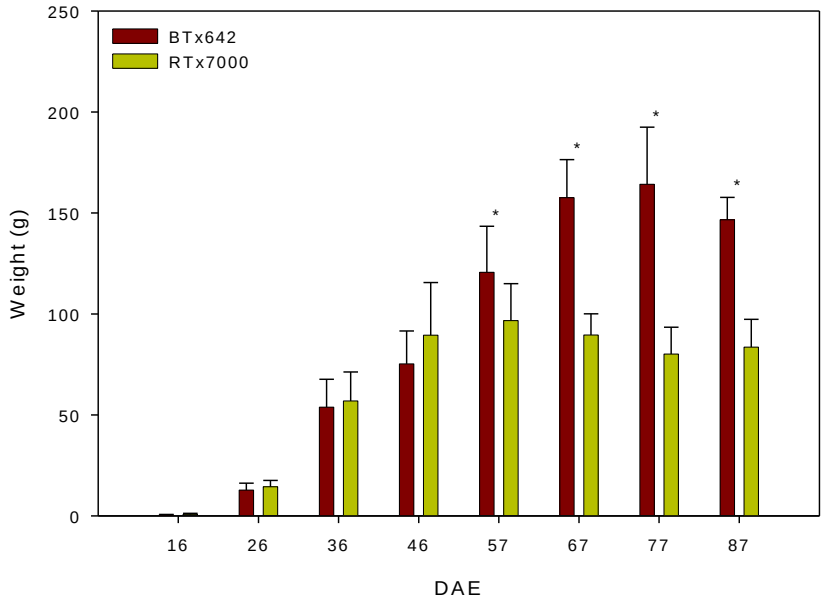


Figure 2.4. BTx642 and RTx7000 total leaf fresh weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at $P<0.05$ are denoted by an asterisk above the bars.

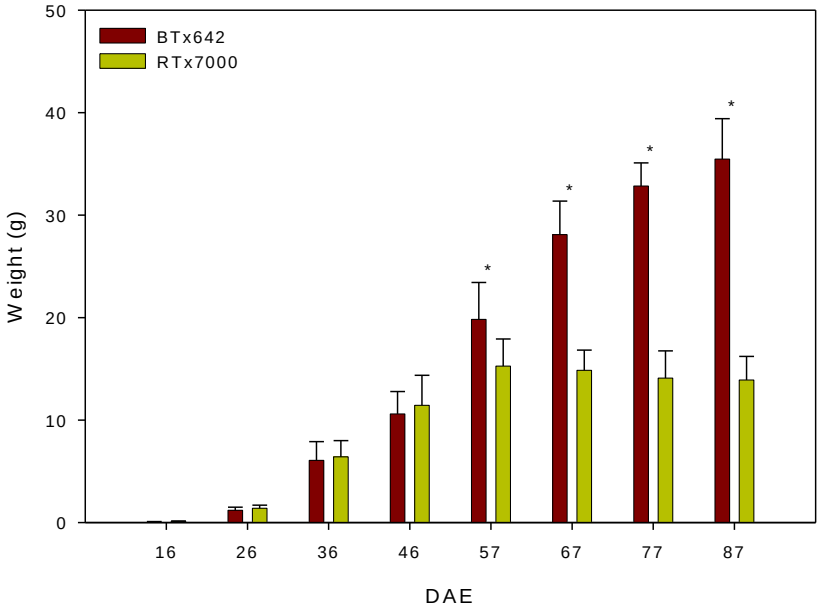


Figure 2.5. BTx642 and RTx7000 total leaf dry weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

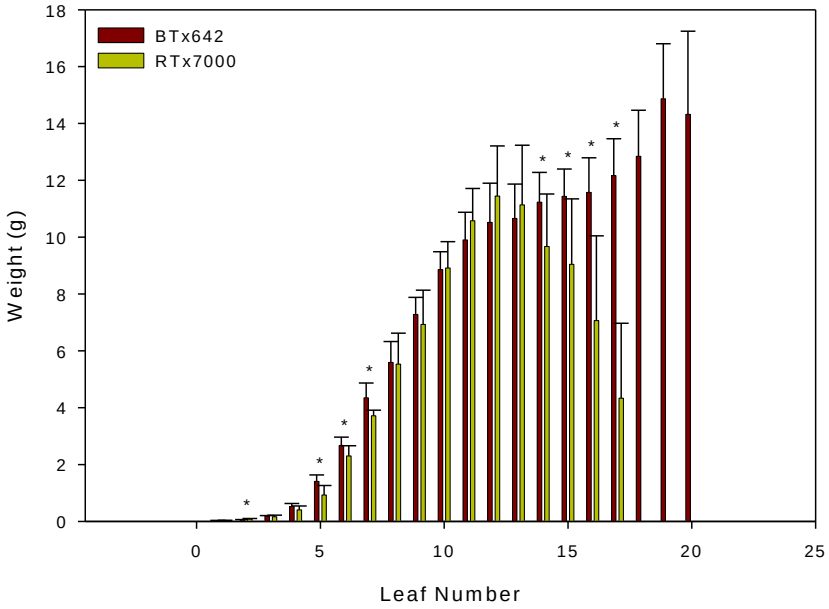


Figure 2.6. BTx642 and RTx7000 individual leaf fresh weight. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

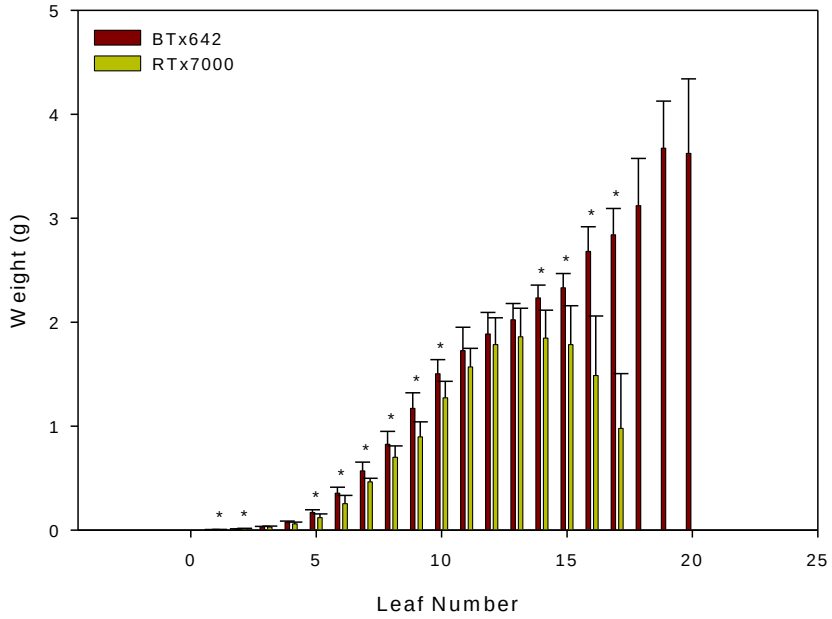


Figure 2.7. BTx642 and RTx7000 individual leaf dry weight. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at $P<0.05$ are denoted by an asterisk above the bars.

Total leaf area of RTx7000 was greater than BTx642 up until ~36 DAE when total leaf area became similar between the two genotypes (Fig. 2.8). By 46 DAE RTx7000 total leaf area was again greater than BTx642 and remained so until a transition point at 67 DAE; thereafter leaf area was similar between the genotypes followed by significantly greater total leaf area in BTx642 for the remainder of the study. The area of individual leaves followed a similar time course (Fig. 2.9). Leaves one through four were similar in area between the two genotypes, and leaves five through seven, eight through nine, and 14-17 showed greater leaf area in BTx642. Greater total leaf area in RTx7000 in the earlier time points can be attributed to more

leaves in RTx7000, and at 57 DAE can be attributed to the rapid rate of leaf expansion between 46-57 DAE in RTx7000. The differences in leaf area during development and between BTx642 and RTx7000 were reflected in differences in both leaf length and width (Fig. 2.10, 2.11). Specific leaf fresh and dry weight (weight/unit area) increased in parallel in both genotypes up to leaf eight (Fig. 2.12, 2.13). At this point specific weight continued to increase in parallel in general, although a few of the upper leaves had greater specific fresh weight in RTx7000 and greater specific dry weight in BTx642.

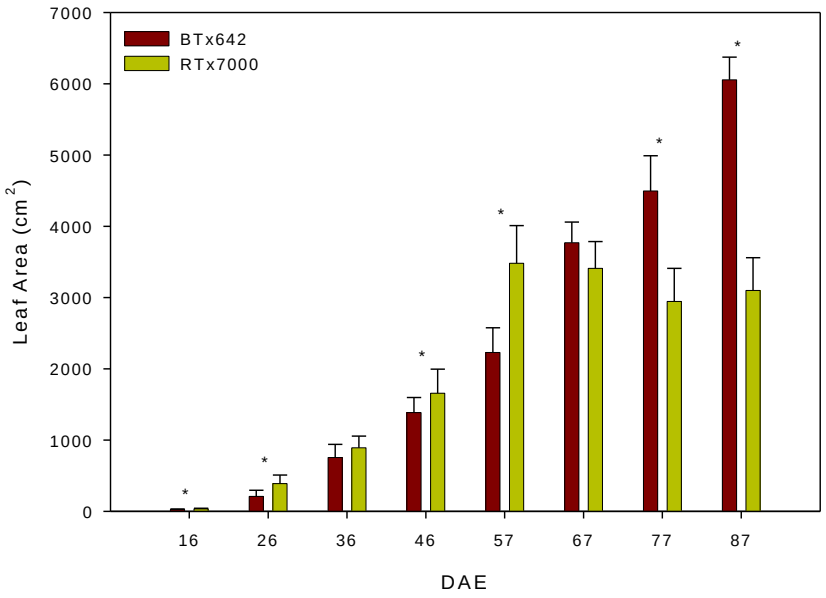


Figure 2.8. BTx642 and RTx7000 total leaf area time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at $P<0.05$ are denoted by an asterisk above the bars.

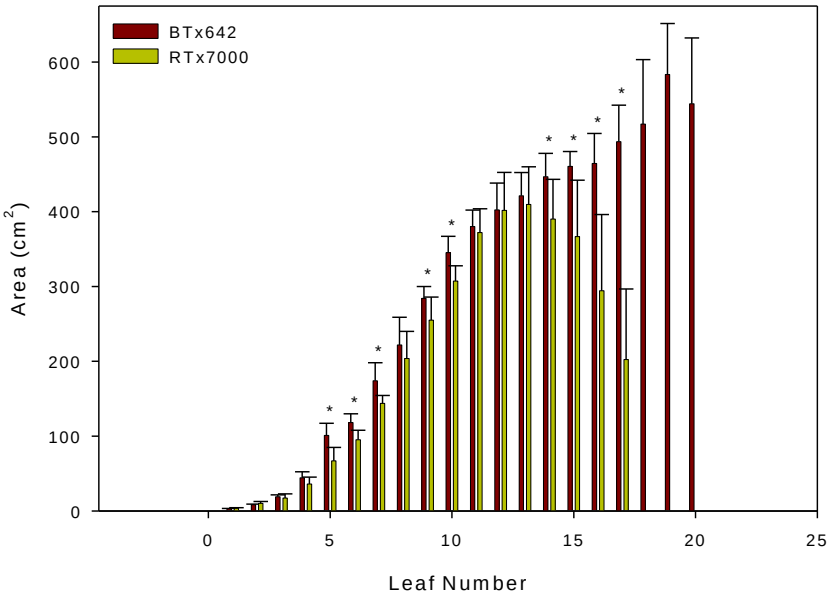


Figure 2.9. BTx642 and RTx7000 individual leaf area. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

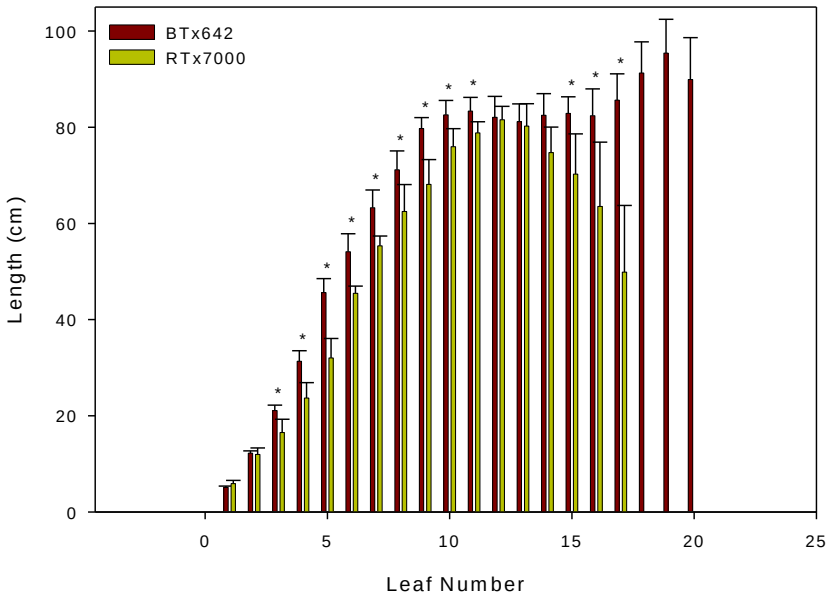


Figure 2.10. BTx642 and RTx7000 individual leaf length. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

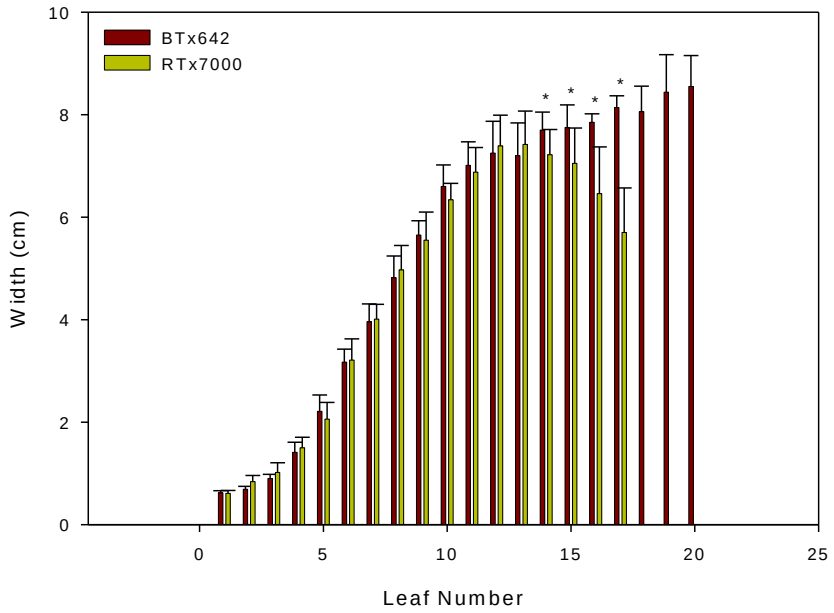


Figure 2.11. BTx642 and RTx7000 individual leaf width. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

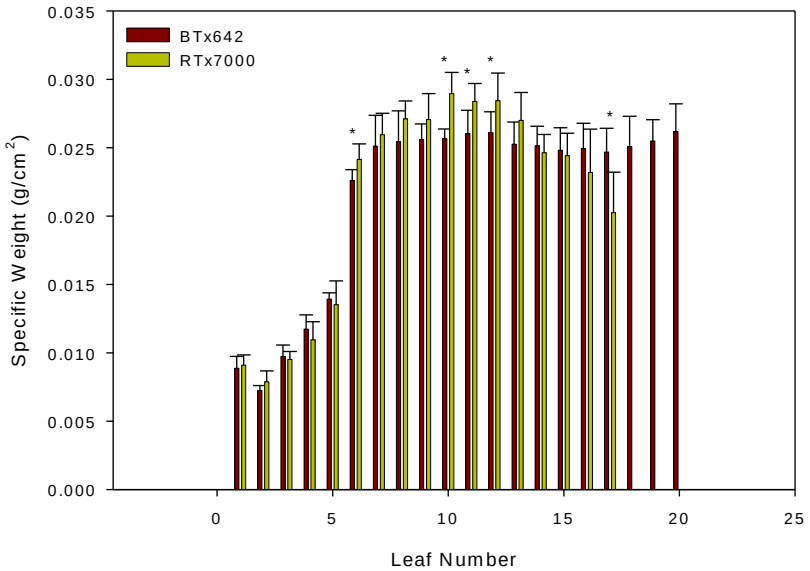


Figure 2.12. BTx642 and RTx7000 individual leaf specific fresh weight. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

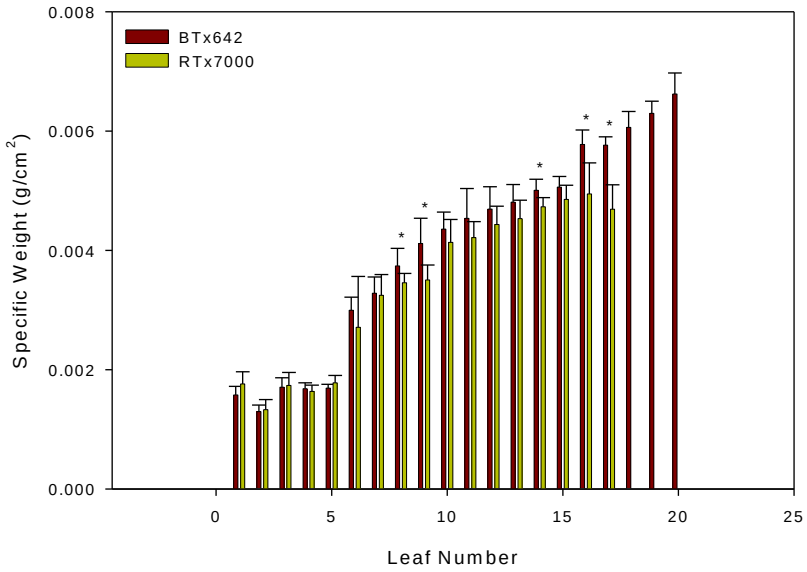


Figure 2.13. BTx642 and RTx7000 individual leaf specific dry weight. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at $P<0.05$ are denoted by an asterisk above the bars.

Stalk, Leaf Sheath, and Stem Traits

Stalk (stem plus leaf sheath) weight increased steadily throughout development in both genotypes until 67 DAE when stalk weight reached a maximum in RTx7000 (Fig. 2.14, 2.15). BTx642 and RTx7000 had similar stalk fresh and dry weights until 57 DAE, with the exception that stalk fresh weight was slightly higher in RTx7000 at 26 DAE and slightly higher in BTx642 at 36 DAE. By 57 DAE stalk dry weight was higher in RTx7000, but this quickly changed as BTx642 maintained higher stalk weight from 67 DAE through anthesis. At 87 DAE, BTx642 had accumulated 54.1 ± 8.5 g whereas RTx7000 had accumulated 18.0 ± 4.4 g in stalk dry weight (Fig. 2.15).

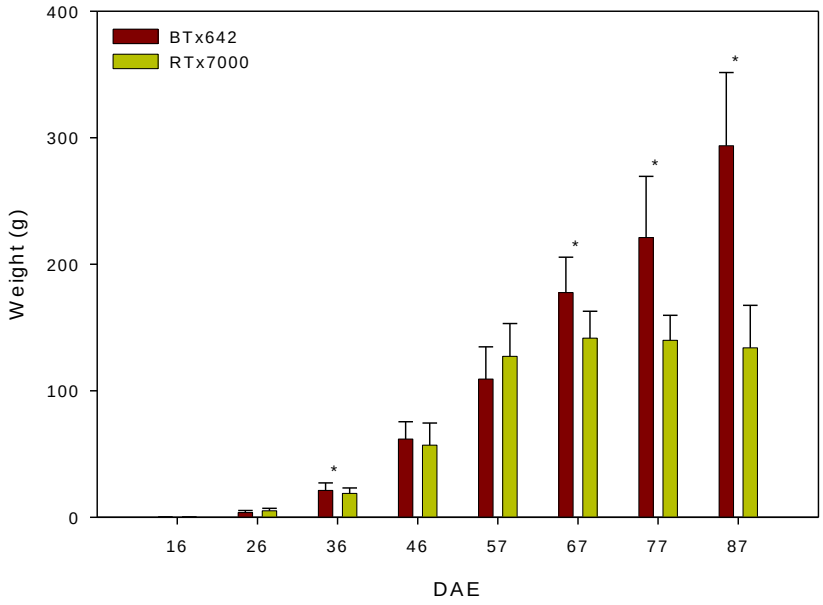


Figure 2.14. BTx642 and RTx7000 stalk fresh weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

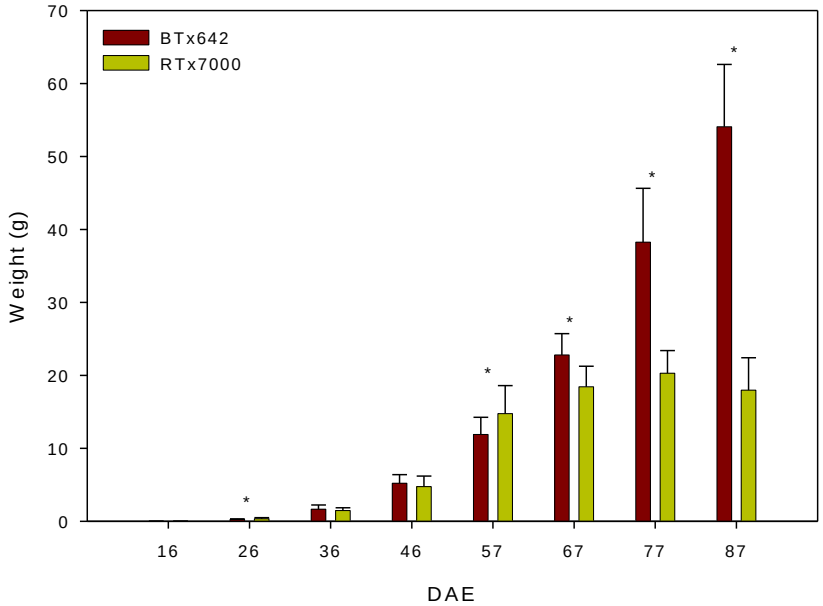


Figure 2.15. BTx642 and RTx7000 stalk dry weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

Stalks can be subdivided into the stem and the leaf sheaths, and differences in these subcomponents can be attributed to the differences observed in stalk weight. RTx7000 had greater leaf sheath weight than BTx642 at 57 DAE, contributing to greater stalk dry weight by RTx7000 at this same time point (Fig. 2.16, 2.17). BTx642 had both higher leaf sheath and stem weights from 67 DAE through the end of the time course, consistent with the higher stalk weights observed for BTx642 during this time frame. Individual leaf sheath weights were significantly higher ($P<0.05$) for BTx642 in most cases (Fig. 2.18, 2.19). At 26 DAE and 57 DAE where total leaf sheath fresh and/or dry weights were greater in RTx7000 (Fig. 2.16, 2.17), these differences can be attributed to the fact that RTx7000 had both more leaves and more fully expanded leaves at these time points.

Stalk weight can also be affected by stalk length and stem length. Stalk length was measured from the base of the stem to the junction between the leaf sheath and leaf blade of the last fully expanded leaf. As shown in Figure 2.20, RTx7000 had greater stem length at 46-67 DAE and greater stalk length at 57-77 DAE. BTx642 was greater in stalk length at 26 DAE and greater in stem length at 87 DAE. Most of the rapid expansion of the stalk is coincident with the timing of anthesis. Anthesis occurred in RTx7000 at ~62 DAE, consistent with the rapid expansion in length of the stalk between 46-57 DAE. Similarly, the most rapid growth in length of the BTx642 stalk occurred between 77-87 DAE, consistent with the observation of anthesis for this genotype at ~85 DAE.

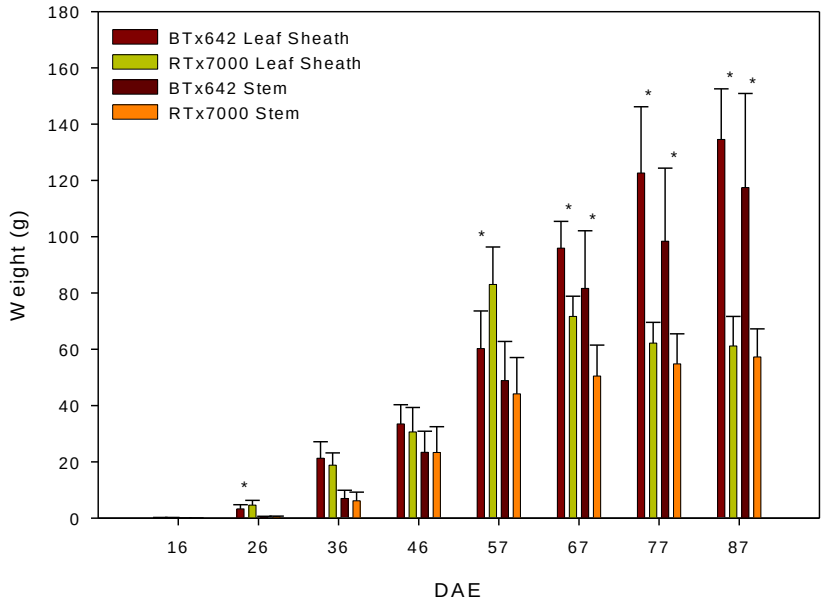


Figure 2.16. BTx642 and RTx7000 leaf sheath and stem fresh weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

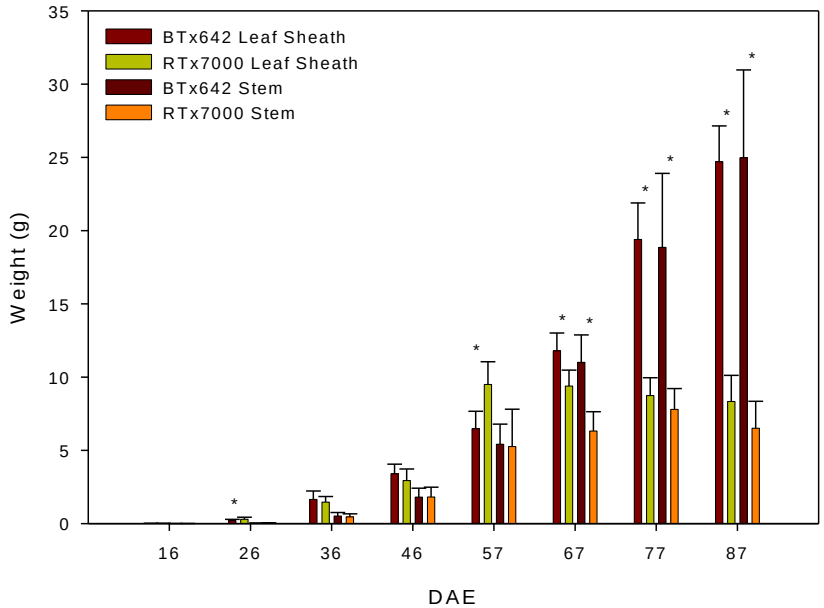


Figure 2.17. BTx642 and RTx7000 leaf sheath and stem dry weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

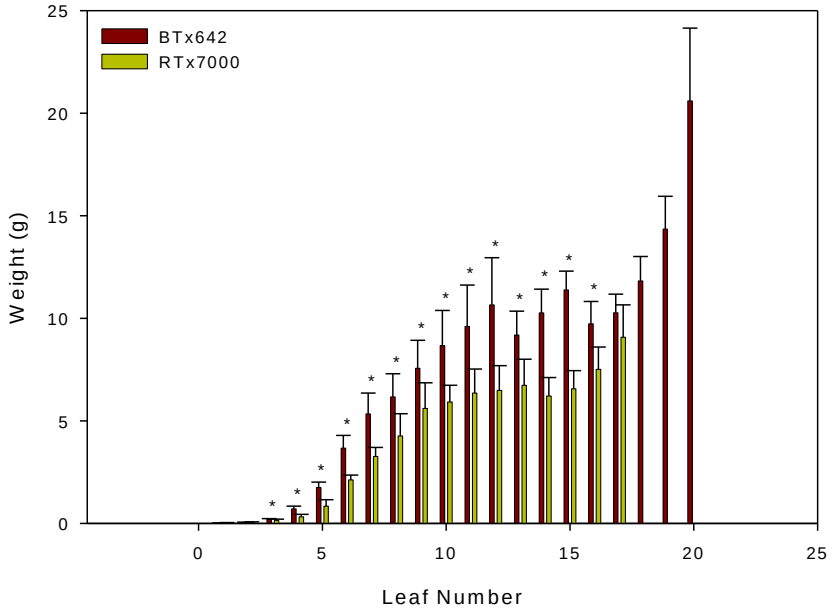


Figure 2.18. BTx642 and RTx7000 individual leaf sheath fresh weight. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

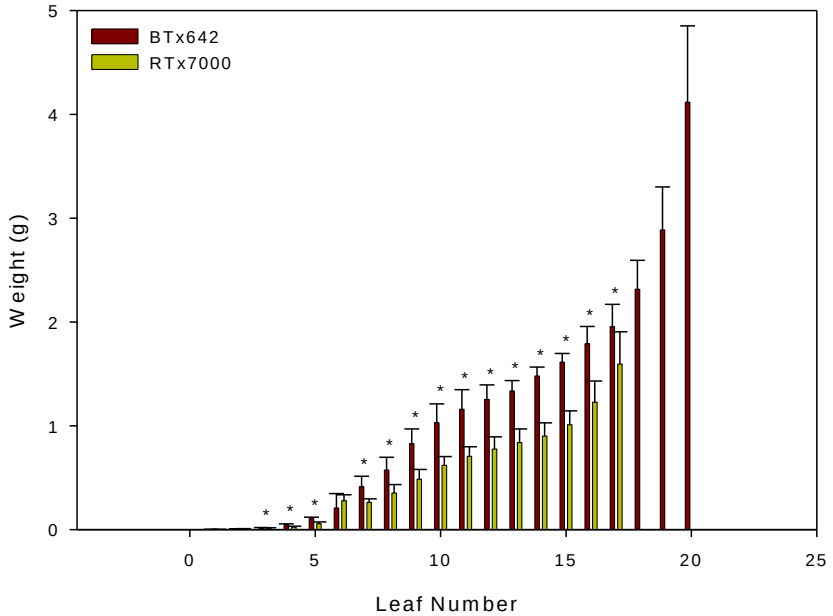


Figure 2.19. BTx642 and RTx7000 individual leaf sheath dry weight. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

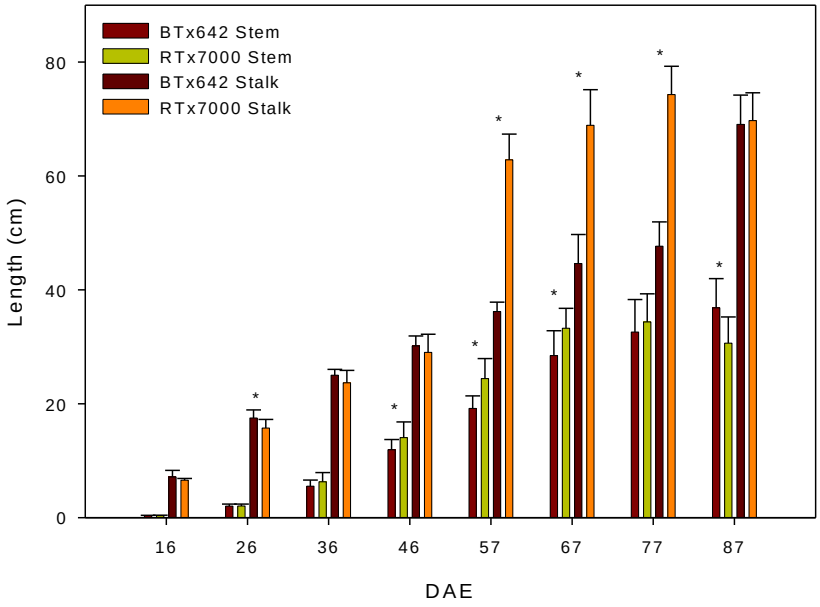


Figure 2.20. BTx642 and RTx7000 stem and stalk length time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at $P<0.05$ are denoted by an asterisk above the bars.

Root Traits

Washed nodal roots from BTx642 and RTx7000 were removed from the root crown, counted, measured in length, weighed, and then scanned during the study for further analysis. The root systems of both genotypes grew in parallel and quickly as they reached a depth of ~40 cm by 16 DAE, ~110 cm by 26 DAE, and by 36 DAE at least one nodal root on average had reached the bottom of the pipe (150 cm). From this point on additional growth in depth of nodal roots at the bottom of the pipe was minimal.

Root fresh and dry weights for both genotypes rose steadily and at approximately the same rate until 57 DAE at which point RTx7000 was close to anthesis (~62 DAE)

(Fig. 2.21, 2.22). From 67 to 87 DAE, RTx7000 root fresh and dry weights declined to 82.2 ± 28.4 g and 9.2 ± 4.0 g, respectively, while BTx642 fresh and dry weights increased to 247.8 ± 34.5 g and 41.4 ± 8.5 g, respectively during the same time frame. Root weight can be affected by the number of nodal roots the genotype produces. By 26 DAE, RTx7000 had produced ~14 nodal roots, an amount significantly higher ($P<0.05$) than the ~12 nodal roots produced by BTx642 (Fig. 2.23). RTx7000 continued to produce significantly more nodal roots than BTx642 through 57 DAE when RTx7000 had ~ 54 nodal roots compared to the ~41 nodal roots produced by BTx642. At this stage in development RTx7000 ceased production of new nodal roots, while BTx642 continued this process, eventually surpassing the RTx7000 total nodal root number from 77-87 DAE. As a consequence of having more nodal roots than BTx642 from 26-57 DAE, RTx7000 also had greater total nodal root length, reaching nearly 40 m in length, an amount significantly larger than the ~30 m in total length BTx642 had reached by the same time point (Fig. 2.24).

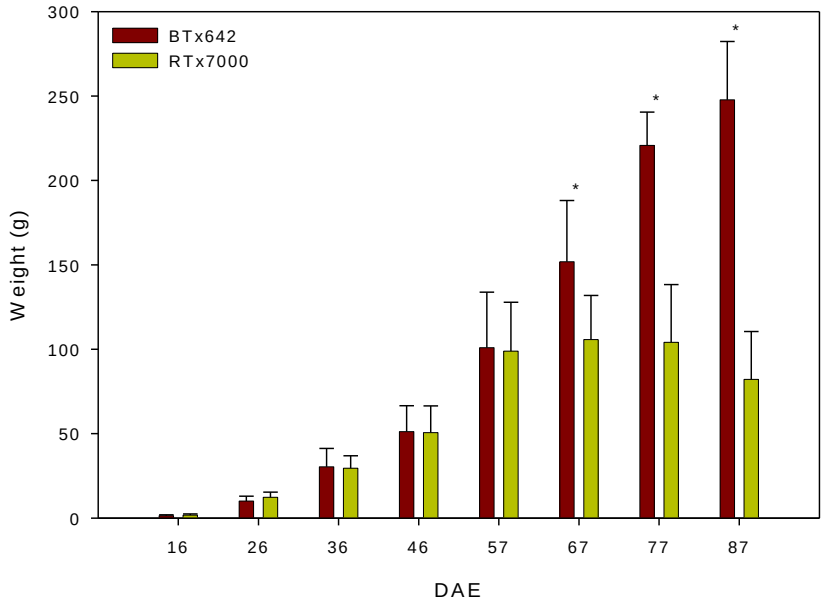


Figure 2.21. BTx642 and RTx7000 root fresh weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

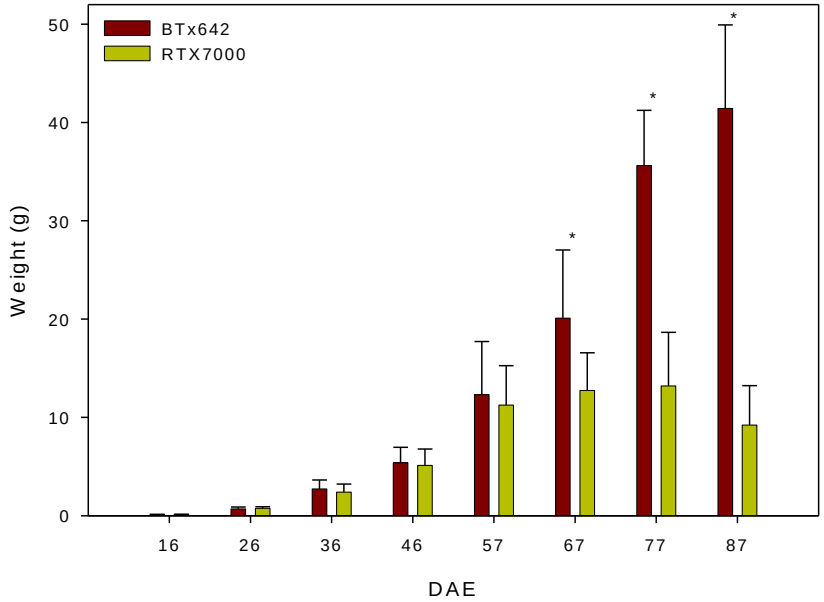


Figure 2.22. BTx642 and RTx7000 root dry weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

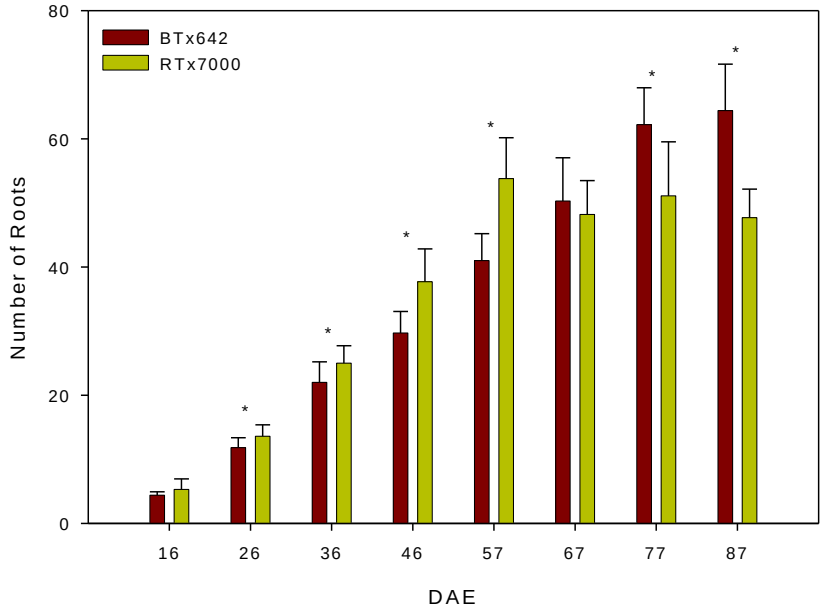


Figure 2.23. BTx642 and RTx7000 total number of nodal roots time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

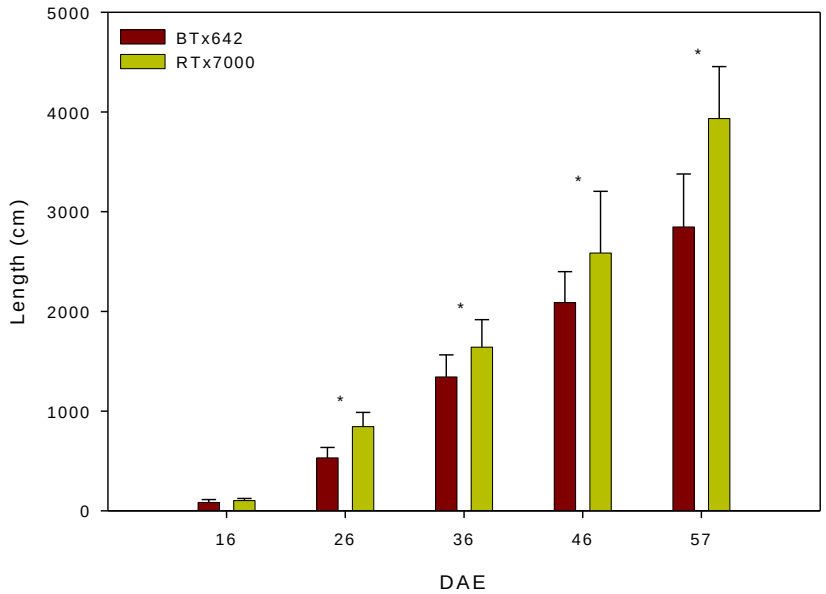


Figure 2.24. BTx642 and RTx7000 total nodal root length time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

To further break down components of the root system, scanned images of BTx642 and RTx7000 root systems were analyzed from 16-57 DAE for total root length, surface area, volume, number of root tips and forks, and average root diameter. Due to the complexity of analyzing large root systems, no detailed analyses were done on root systems past 57 DAE. Total root length, surface area, and volume steadily increased for both genotypes (Figs. 2.25-2.27). RTx7000 was significantly greater with respect to these traits at 26 DAE. By 57 DAE, both genotypes had developed roots systems of total length ~350-400 m, total surface area ~0.48-0.50 m^2, and a total volume of ~60 cm^3. The number of root forks was significantly greater in RTx7000 at 16-26 DAE, but not at any other time points with respect to the total number of root forks or tips (Fig. 2.28, 2.29). Both genotypes had produced ~280,000-320,000 root forks and ~200,000-220,000 root tips by 57 DAE. Average root diameter was significantly higher for RTx7000 at 16 DAE, but declined and then remained fairly constant with respect to BTx642 for later growth stages (Fig. 2.30).

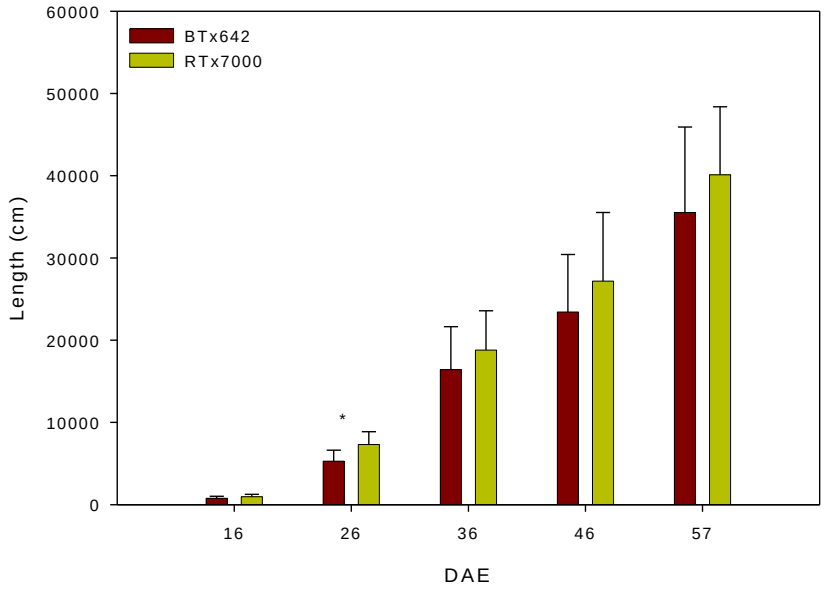


Figure 2.25. BTx642 and RTx7000 total root length time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

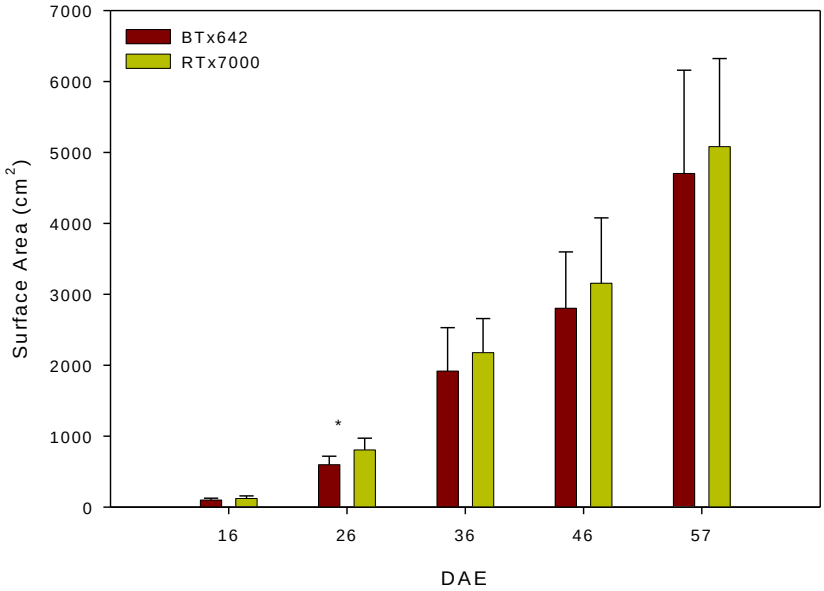


Figure 2.26. BTx642 and RTx7000 total root surface area time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

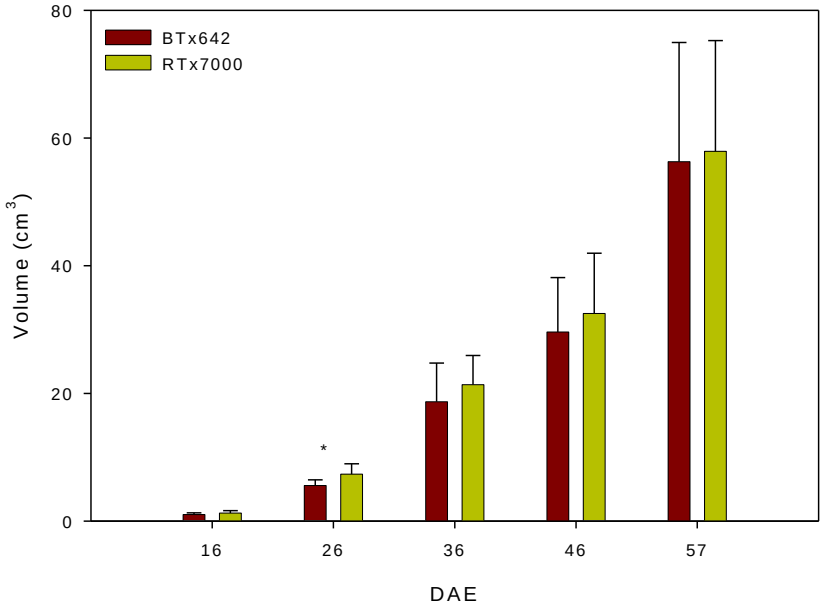


Figure 2.27. BTx642 and RTx7000 total root volume time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

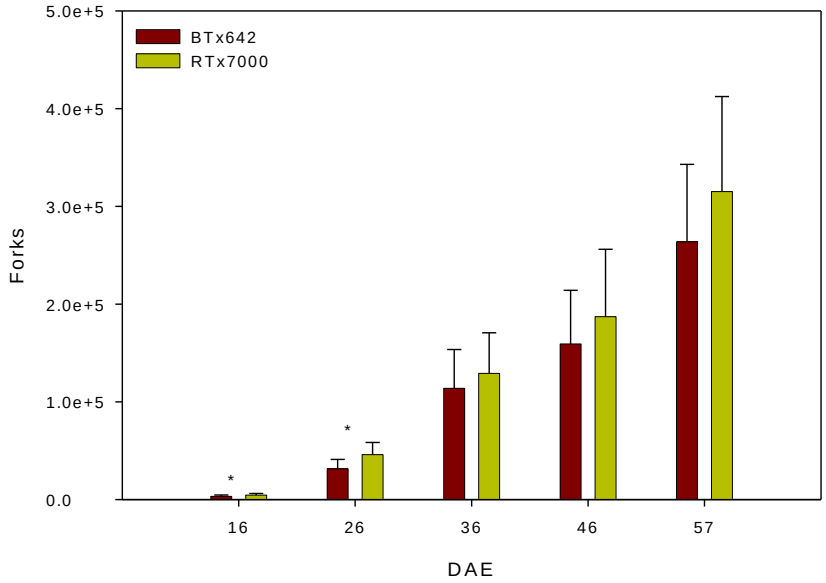


Figure 2.28. BTx642 and RTx7000 total number of root forks time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

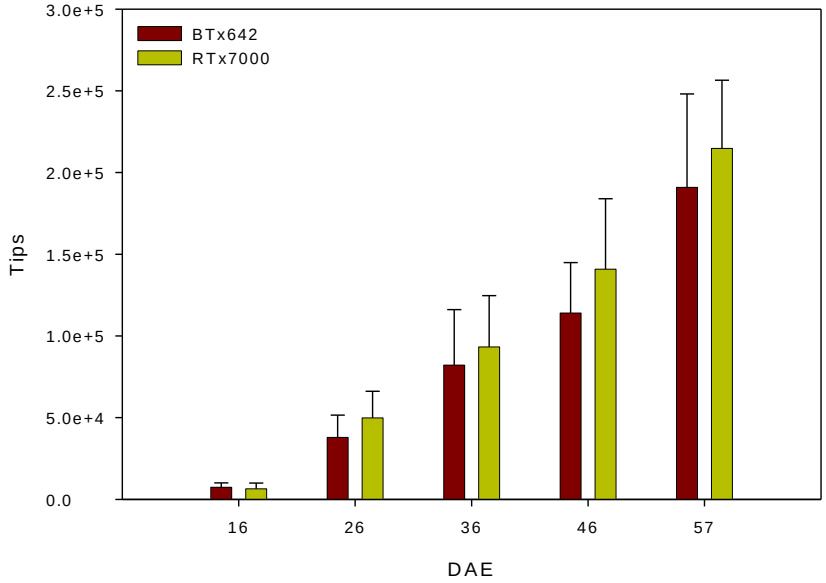


Figure 2.29. BTx642 and RTx7000 total number of root tips time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

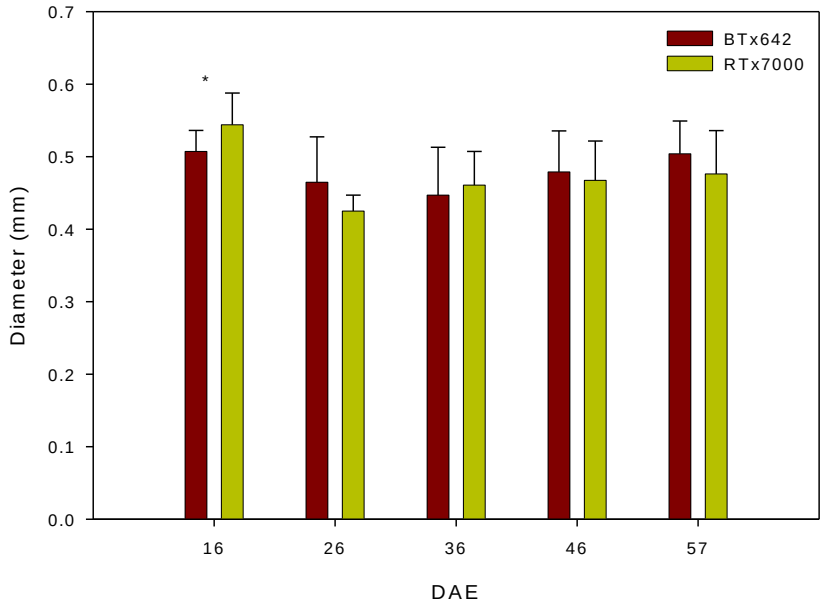


Figure 2.30. BTx642 and RTx7000 average root diameter time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

Total Biomass Traits

Through harvesting both roots and shoots of BTx642 and RTx7000 to 87 DAE the entire growth and development of the two genotypes could be analyzed with regard to total biomass production. Both genotypes produced fairly equal amounts of total fresh weight biomass through 57 DAE, at which point RTx7000 total fresh biomass leveled off and BTx642 total fresh biomass continued to increase (Figure 2.31). At the same point total dry biomass continued to increase for both genotypes, with BTx642 maintaining significantly greater total dry biomass from 67-87 DAE (Figure 2.32). The difference between genotypes starting at 67 DAE is likely due to the timing of anthesis for RTx7000. Total fresh weight leveling off in RTx7000 is consistent with total shoot and root fresh weight leveling off at this time point (Fig. 2.1, 2.21) and is also consistent with panicle development during this time frame, which is likely responsible for the overall increase in shoot total dry weight from 67-87 DAE as the panicle acts as a sink for assimilate (Fig. 2.2).

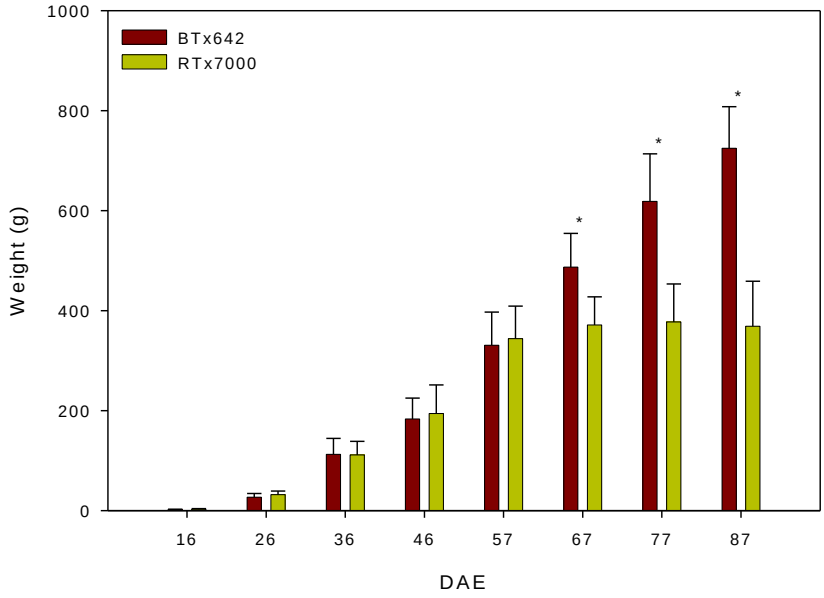


Figure 2.31. BTx642 and RTx7000 total root and shoot fresh weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

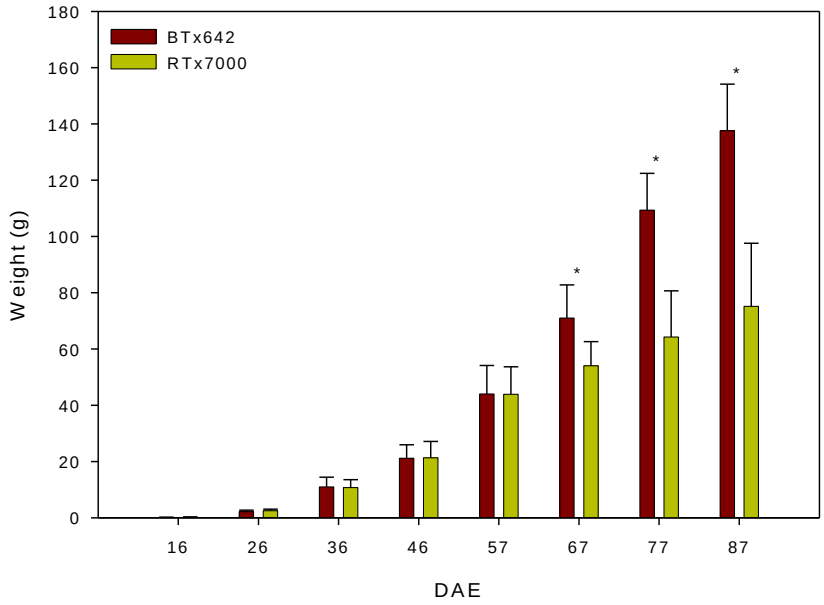


Figure 2.32. BTx642 and RTx7000 total root and shoot dry weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at P<0.05 are denoted by an asterisk above the bars.

From germination to 16 DAE, both genotypes differentially developed root systems, as more than twice of BTx642's and more than 1.5 times RTx7000's fresh weight total biomass was allocated to the root system (Fig. 2.33). Dry weight allocation to the root system was high as well with root:shoot dry weight ratios approaching 1.2 for BTx642 and 1.0 for RTx7000 (Figure 2.34). Past 16 DAE, root:shoot ratios declined and held steady at ~0.4 for both genotypes with regard to fresh and dry weights. Between 77 to 87 DAE the allocation of fresh and dry weight became significantly different between RTx7000 and BTx642, consistent with reallocation of assimilate from root sinks to the panicle sink.

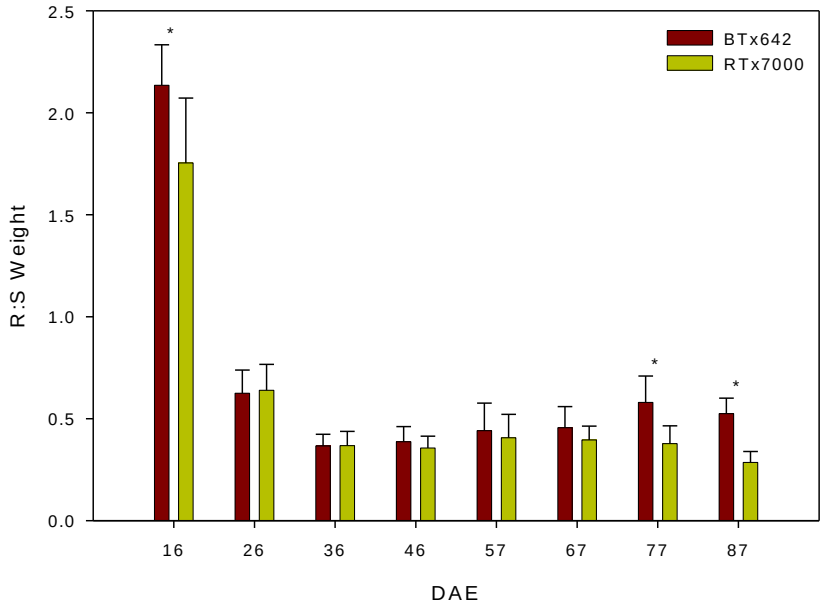


Figure 2.33. BTx642 and RTx7000 root:shoot fresh weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at $P<0.05$ are denoted by an asterisk above the bars.

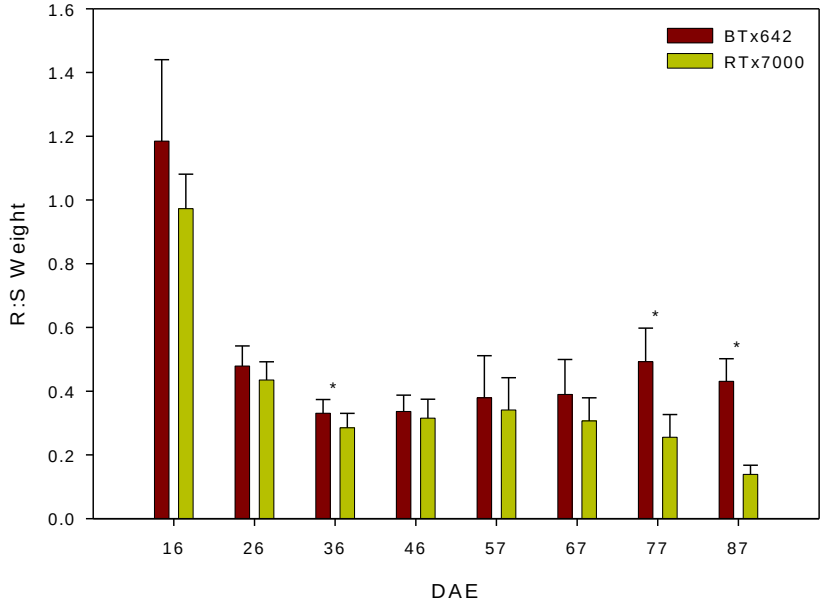


Figure 2.34. BTx642 and RTx7000 root:shoot dry weight time course. Data are shown as means ± standard deviation. At a given time point, data were subjected to a *t*-test to determine significant differences between the means. Means different at $P<0.05$ are denoted by an asterisk above the bars.

Discussion

The time course of BTx642 and RTx7000 growth and development was analyzed and compared in order to identify differences in phenology and partitioning of growth between leaves and stalks that might be related to post-flowering drought tolerance. BTx642 and RTx7000 were grown under decreasing day length conditions from 16-46 DAE in an attempt to create an environment that induced both genotypes to flower at approximately the same time. However, even under the modified day length conditions, RTx7000 flowered ~23 days earlier than BTx642. This difference in time to floral initiation could be due to variation in the length of the juvenile phase, a period when

plants are photoperiod insensitive, or differences in other processes that modulate flowering time after completion of the juvenile phase. Quinby (1967) estimated that the juvenile phase in the grain sorghum genotypes he analyzed ranged from ten to fourteen days after emergence. Experiments were subsequently carried out to explore the genetic and physiological basis of differences in flowering time observed in BTx642 and RTx7000 in greater detail (Chapter V).

During the first 46 DAE BTx642 and RTx7000 followed similar time courses with respect to leaf appearance rate and time to full leaf expansion rate; however, RTx7000 produced and expanded approximately one to two more leaves per time point than BTx642 during this period. Between 46-57 DAE RTx7000 produced and expanded its remaining leaves, while BTx642 continued to produce leaves through 67 DAE and had finished expanding its flag leaf by 87 DAE. Leaf senescence in the two genotypes started at 36 DAE, with each genotype incurring a loss of the first five leaves by 67 DAE. The rate of subsequent leaf senescence then slowed and by 87 DAE both genotypes had senesced about six leaves. Overall, the rate of leaf production and time course of leaf senescence was similar for BTx642 and RTx7000 until RTx7000 was approaching anthesis between 46-57 DAE. However, BTx642 consistently produced larger leaves that contained more dry weight than RTx7000 through most of development. A small cohort of leaves (11-13) was not significantly different in size and weight between genotypes in this study. In other environments such as under field conditions in 2008-2010 (Chapter VII), these leaves were larger in RTx7000, fewer

leaves were produced, and days to flower were shorter for BTx642, thus illustrating the effect environment can play on leaf size.

BTx642 produced approximately three to four more leaves than RTx7000 because floral initiation occurred later in BTx642. The size and weight of leaves 14-20 were distinctly larger and heavier than the last leaves developed by RTx7000. Additionally, the progression of leaf size and weight from small in early stages of growth to a maximal point approaching anthesis, followed by a decline as the panicle is developing, as seen in RTx7000, was not observed in BTx642. Instead, leaf size continued to rise for BTx642 through the expansion of the flag leaf. This response may be tied to flowering time, as the approximate 23 day delay in flowering time relative to RTx7000 allowed BTx642 to remain in the vegetative phase longer and therefore produce more leaves. If this effect is entirely caused by flowering time, leaf size parameter QTL for the RIL population derived from BTx642 and RTx7000 should co-align with QTL for flowering time; if these traits are controlled by separate genetic loci, then it would be possible to create genotypes that have similar flowering time but different kinetics of leaf area development and total leaf area at anthesis that would allow one to assess the impact of this difference on rate and extent of water use and drought tolerance. Analysis of RIL populations and QTL mapping experiments described in subsequent chapters will address some of these possibilities.

Analysis of stalk, stem, and leaf sheath traits during development revealed differences in the time course of growth and development of these organs in BTx642 and RTx7000. In sync with leaf development, accumulation of weight in stalks (stem plus

leaf sheath), stems, and leaf sheaths was greater in BTx642 than RTx7000 in later stages of growth. Stalk length was greater in BTx642 than RTx7000 at 26 DAE, whereas RTx7000 stem length was greater than BTx642 from 46-67 DAE and stalk length was greater from 57-77 DAE when upper stem elongation accelerated in RTx7000 just prior to anthesis. Overall the larger leaf weight observed in BTx642 relative to RTx7000 may enable the plant to produce more photosynthate that can be assimilated into the stalk. It has been noted that BTx642 has increased stalk-lodging resistance compared to RTx7000 and this trait may be related to increased stalk biomass allocation in this genotype. The higher allocation of biomass to stems in BTx642 may also provide a sink for carbon (sugars) that can be used for grain filling following anthesis. In fact BTx642 is known to accumulate more stem sugar than RTx7000, a fact that was confirmed in this study (data not shown) (Sowder et al., 1997).

One might hypothesize that a plant adapted to drought prone environments would have small leaves so that when water-limiting conditions occur transpirational water loss would be reduced due to a smaller evaporative leaf surface. An examination of total leaf area over time (Fig. 2.8) showed that RTx7000 had greater total leaf area than BTx642 from 16-26 DAE and 46-57 DAE. BTx642 only exceeded RTx7000 in leaf area once RTx7000 stopped producing leaves and entered anthesis. If BTx642 and RTx7000 had reached anthesis at the same time, total leaf area of RTx7000 likely would have been greater than BTx642.

It has been proposed that post-flowering drought tolerant plants could compensate for decreased leaf area by increasing the leaf weight per area (specific leaf

weight). One way this could be achieved would be by producing thicker leaves. Thicker leaves would have a greater volume that could potentially increase the amount of chlorophyll per leaf surface area, and hence allow a plant with smaller leaf area to fix as much carbon as a plant with thinner leaves and a larger surface area. However, BTx642 and RTx7000 leaf fresh and dry specific weights were not significantly different for leaves one through seven. These leaves correspond to the five to seven leaves initiated during embryo and seed development (Reznik, 1934; Tarr, 1962). Specific leaf weights for the upper leaves were fairly uniform for both genotypes, with RTx7000 having a few leaves that had higher specific leaf fresh weight and BTx642 having a few leaves with higher specific leaf dry weight. The differences in this parameter in leaves 18-20 were likely due to differences in flowering time and the fact that RTx7000 did not produce these leaves.

A plant with a large root system may be better able to tolerate a water-limiting environment than a plant with a smaller root system, if the system is large with respect to the volume of soil it penetrates, has an abundance of surface area (lots of root hairs) per unit volume of soil, and is deep in the soil profile to extract deep water. Both BTx642 and RTx7000 appear to have the capability to build large root systems, relatively speaking, as evidenced by the parallel accumulation in root weight through 57 DAE (Fig. 2.19. 2.20). At this time point RTx7000 entered anthesis and growth in root weight diminished, likely because sink strength was high in the developing panicle, in effect reducing the amount of assimilate available for root growth. BTx642 however continued producing root biomass through the rest of the time course, first surpassing RTx7000 at

67 DAE. Had flowering times been closer between the genotypes, differences in root biomass may not have been observed. Interestingly, even though root biomass was similar between the two genotypes through 57 DAE, RTx7000 produced significantly more nodal roots and had a correspondingly greater nodal root length than BTx642 from 26-57 DAE. This is an indication that under these conditions RTx7000 is developing a complex root system faster than BTx642, and this may contribute to its previously observed pre-flowering drought tolerance with respect to shoots under field conditions (Duncan et al., 1981; Rosenow et al., 1983).

In conclusion, several parameters including flowering time, height, shoot biomass, leaf size, total leaf area, root biomass, and root size varied in BTx642, a post-flowering drought tolerant genotype, and RTx7000, a post-flowering drought susceptible genotype, under pre-anthesis well-watered conditions. The relationship between variation in these traits and stay-green post-flowering drought tolerance as well as shoot biomass and yield will be examined in subsequent chapters through analysis of a RIL population derived from these genotypes and QTL analysis.

CHAPTER III

BTx642/RTx7000 GENETIC MAP

Introduction

The stay green drought tolerance phenotype refers to the retention of photosynthetically active green leaf area during post-flowering drought. For sorghum, when moisture stress occurs during grain filling, yield is reduced and charcoal rot and lodging ensue (Rosenow and Clark, 1981). In Type A and B stay green plants (Thomas and Howarth, 2000), however, the plant remains green and continues to fill grain under drought conditions. Moreover, under well-watered conditions yield is not compromised (Borrell et al., 2000). Physiologically, relative to senescent genotypes, stay green genotypes have increased basal stem sugars (Duncan, 1981), cytokinins (McBee, 1984), nitrogen content (Borrell et al., 2000), and improved transpirational efficiency (Borrell et al., 2000).

Genetic maps are required to identify QTL and alleles of genes responsible for variation in traits such as stay green. Several groups have constructed genetic maps and conducted QTL analysis using sorghum populations that segregate for stay green. Sources of stay green that have been studied to date include SC56, from the caudatum race in Sudan (Kebede et al., 2001), E36-1, from the caudatum-guinea race in Ethiopia (Haussmann et al., 2002), and of most relevance to the current work, a durra sorghum BTx642 (formerly B35), from Ethiopia (Tuinstra et al., 1997; Crasta et al., 1999; Subudhi et al., 2000; Xu et al., 2000). Xu et al. (2000) mapped four stay green loci using a RIL population derived from a cross between BTx642 (stay green) and RTx7000

(senescent) grown under dryland conditions: *Stg1* and *Stg2* on LG-03, *Stg3* on LG-02, and *Stg4* on LG-05. Stay green phenotypic data from Xu et al. (2000) was used in the current study to localize the previously mapped stay green QTL onto a higher resolution genetic map for this population that consists of Digital Genotyping (DG) markers. QTL for a wide range of other traits were also mapped in this population in the present study and these QTL will be discussed in relation to stay green, shoot biomass, and yield QTL in subsequent chapters.

Materials and Methods

Digital Genotyping Template Preparation[1, 2]

Ten plants were harvested for each of 90 recombinant inbred line (RIL) progeny as well as from the parental lines (BTx642, RTx7000) at eight DAE. Tissue from each line was pooled and genomic DNA isolated using the FastPrep standard protocol (MP). Purified DNA was quantified using the dsDNA BR assay kit (Invitrogen) with a Qubit fluorometer (Invitrogen). One 500 ng aliquot of DNA from each line was placed in a separate well of a 96-well PCR plate and the total volume of each well was brought up to 16.8 µl with dH_2O. The DNA was digested with *NgoM*IV (New England BioLabs), a restriction enzyme that recognizes the six base sequence GCCGGC. Each DNA was digested using the following reaction conditions: 16.8 µl of DNA, 2.0 µl of Buffer 4 (New England BioLabs), 0.2 µl of 100x BSA at 10 mg/ml (New England BioLabs), and two units of *NgoM*IV.

[1]Template was prepared by Daryl Morishige, Ph.D.
[2]Patent pending (Mullet and Morishige, 2009).

The reaction was allowed to proceed at 37° C for four hours. Following DNA digestion, adapters containing 24 different ID sequences were ligated to the termini generated by *NgoM*IV. Sets of 24 DNAs containing different ID sequences were pooled. Each adapter contained a unique four base ID index sequence to be used for downstream computer processing to associate DNA sequences obtained from the Illumina Genome Analyzer II to the correct progeny RIL. The adapter ligation reaction was carried out as follows. To the *NgoM*IV-digested DNA, 2.5 µl of 10x ligase buffer (Promega), 1.0 µl of adapter B (5 µM) (Illumina), 1.5 units of T4 DNA ligase (Promega) and dH_2O to 25.0 µl were mixed and then incubated at 20° C for four hours. The reaction was stopped by heating to 65° C for 15 minutes. Next, the individual reactions were pooled to yield 4 pools of 24 samples each. The DNA in each pool was ethanol precipitated by addition of 60 µl of 3.0 M sodium acetate and two volumes of ethanol. The solution was mixed and incubated on ice for 30 minutes. DNA was pelleted by spinning at 12,000 x g for ten minutes at 4° C. The supernatant was decanted and the pellet washed with 1.0 ml of 70% cold ethanol. After air drying for 10 minutes, the pellet was resuspended in 100.0 µl TE buffer and then subjected to shearing. The DNA was sheared with a Bioruptor (Diagenode) to both further reduce the size of the fragments and to produce fragments of similar length (~150-300bp). DNA shearing was accomplished by cooling the Bioruptor water bath with ice and then running the instrument for 20 minutes in a 15 second on-15 second off cycle using the "low" setting and a power level of four. After shearing, 180 µl of AMPure XP solution (Beckman-Coulter) was added to each tube and the DNA was purified using the standard AMPure XP Bead Extraction protocol (Beckman-Coulter).

The sheared DNA was electrophoresed on a 2.0% agarose gel at 100 volts for two hours and fragments in the 200-300 bp size range were excised from the gel and purified using the Qiaquick Gel Purification Kit standard protocol (Qiagen). Purified DNA was eluted in 35.0 µl of Buffer EB (Qiagen). In preparation for ligation of adapters to DNA termini generated by shearing, 5' overhangs were filled in using *Bst* Polymerase (New England BioLabs). Two µg of purified DNA in 35.0 µl of Buffer EB (Qiagen) were mixed with 5.0 µl of 10x ThermoPol Buffer (New England BioLabs), 1.0 µl of 10 mM dNTP mix (New England BioLabs), 0.5 µl 100x BSA (10 mg/ml) (New England BioLabs), 20 units of *Bst* Polymerase, and 6 µl of dH_2O. The reaction was incubated at 65° C for 30 minutes and then the DNA purified using the Qiaquick PCR Purification Kit standard protocol (Qiagen). Next, the remaining sheared ends of the fragments were made blunt via the New England BioLabs Quick Blunting Kit. Two µg of purified DNA were mixed with 2.5 µl of 10x Blunting Buffer (New England BioLabs), 2.5 µl of 1 mM dNTP mix (New England BioLabs), 1.0 µl of Blunting Enzyme, and dH_2O to 25.0 µl. The reaction was incubated at room temperature for 30 minutes and then the DNA purified using the Qiaquick PCR Purification Kit standard protocol (Qiagen). Purified fragments were adenylated on the 3'-ends to provide specificity for ligation of a T-tailed adapter. Adenylation was carried out by mixing the purified DNA fragments with 5.0 µl of Buffer 2 (New England BioLabs), 10.0 µl of 250 µM dATP (New England BioLabs), 10.0 units of Klenow 3'-5' Exo- (New England BioLabs), and dH_2O to 50.0 µl. The reaction was incubated at 37° C for 30 minutes and then purified using the Qiaquick PCR Purification Kit standard protocol (Qiagen). Ligation of a T-tailed adapter

proceeded by mixing the purified DNA fragments with 5.0 µl of 10x T4 DNA Ligase Buffer (Promega), 1.0 µl of 10 µM adapter, and 3.0 units of T4 DNA Ligase (Promega). The ligation reaction was performed at 20° C for 4 hours, followed by a 65° C heat treatment for 15 minutes to inactivate the enzyme. The DNA was purified using the standard AMPure XP Bead Extraction protocol (Beckman-Coulter). Purified DNA was PCR-amplified to generate fragments containing an *NgoM*IV site adapter on one end and a T-tailed adapter on the other end. The primer specific to the *NgoM*IV site was biotinylated to allow for separation of fragments containing an *NgoM*IV adapter on one end and a T-tailed adapter on the other end from fragments containing a T-tailed adapter on both ends. The reaction was performed by mixing 125.0 ng of purified DNA, 50.0 µl of 5x Phusion HF Reaction Buffer (New England BioLabs), 10.0 µl of 10 mM dNTPs (New England BioLabs), 5.0 µl of a 10 µM mix of forward and reverse primers, 5.0 units of Phusion DNA Polymerase (New England BioLabs), and dH_2O to 250.0 µl. PCR was performed by first denaturing the DNA at 98° C for 30 seconds, then performing 20 cycles of the following sequence: 98° C for 10 seconds, 58° C for 30 seconds, and 72° C for 30 seconds. Products were polished at 72° C for 10 minutes. The products were purified using the Qiaquick PCR Purification Kit standard protocol (Qiagen) and eluted in 55.0 µl of EB Buffer (Qiagen). Fragments containing an *NgoM*IV adapter on one end were separated from fragments containing the T-tailed adapter at both ends using Dynabeads (Invitrogen). Dynabeads were prepared for addition of DNA by adding 50.0 µl of Dynabeads to a microfuge tube and then spinning at 200 x g for 10 seconds to collect the solution on the bottom of the tube. Each tube was then placed in a magnetic

stand for 1 minute to capture the Dynabeads. The Dynabeads were washed two times with 300.0 µl of binding and washing buffer (5.0 mM Tris (pH 7.5), 0.5 mM EDTA, and 1.0 M NaCl) and then recaptured by placing in a magnetic stand for 1 minute. The washed beads were resuspended in 100.0 µl of binding and washing buffer. Amplified DNA fragments were added to each tube of Dynabeads and incubated at room temperature for 20 minutes with occasional mixing. The beads were captured by placing the tubes in a magnetic stand for 1 minute and then washing with 300.0 µl of binding and washing buffer four times and then with 300.0 µl of dH_2O two times. Single-stranded products were isolated by first washing the DNA-coated beads with 100.0 µl of 1x SSC and then resuspending the beads in 50.0 µl of 1x SSC and incubating at 95° C for 5 minutes. The tubes were placed in a magnetic stand for 1 minute to capture the beads. The supernatant (containing the non-biotinylated strands) was transferred to a tube and the extraction was repeated an additional time. Products were purified with the Qiaquick PCR Purification Kit standard protocol (Qiagen) and eluted in 35.0 µl of Buffer EB (Qiagen). Final PCR was performed to introduce bridge amplification sequences to the ends of the fragments. In this reaction, 50.0 ng of purified ssDNA, 20.0 µl of 5x Phusion Buffer (New England BioLabs), 2.0 µl of 10 µM forward and reverse primer mix, 2.0 units of Phusion DNA Polymerase (New England BioLabs), and dH_2O to 100.0 µl were mixed and subjected to PCR as above. PCR products were purified using the Qiaquick PCR Purification standard protocol (Qiagen) and were eluted in 50.0 µl of Buffer EB (Qiagen). Each of four pools of 24 DNAs was quantified and then run on a Genome Analyzer II using standard parameters (Illumina).

Sequence Analysis and Digital Genotype (DG) Marker Discovery[3]

Image analysis and base calling was performed using Illumina's Pipeline V1.5 sequence analysis software to produce text files containing 76 bp sequences from each lane of the flow cell that passed Illumina's quality filtering. Quality-filtered DNA sequence data was then processed using a series of scripts written in the python programming language. First the 5' end of each sequence was screened for a 4 bp ID tag adjacent to the *NgoM*IV partial restriction sequence. All sequences containing an ID index and partial restriction site were then sorted into individual files based on the progeny or parental line associated with the given index ID. The 4 bp ID sequence was then removed from each sequence and the remaining 72 bp of genomic DNA was clustered to produce a file of unique sequences for each progeny or parental line. Each individual sequence was named according to the line from which it originated as well as for the number of times the given sequence was obtained. Those sequences that were obtained 3 or more times from a given line were than aligned to the BTx623 genome sequence by BLASTN analysis using short sequence parameters (http://www.ncbi.nlm.nih.gov/blast/producttable.shtml#tab31) (P. Klein, in prep.). Sequences that aligned to the BTx623 genome were manually examined to identify sequences that aligned to a unique position within the genome. Sequences that aligned to 2 or more locations within the genome at the same e-value or identity level were labeled as 'repetitive' and removed from further analysis.

[3]Bioinformatic analysis and resulting DG marker discovery were conducted by Patricia Klein, Ph.D.

Only those 72 bp *NgoM*IV-derived sequences that either aligned to 1 location only or those in which the 2nd best location had at least 2 miss matches as compared to the top location were used for analysis of DNA polymorphisms to distinguish BTx642 from RTx7000. A custom script written in the Perl programming language was used to identify polymorphic sequences between BTx642 and RTx7000 by examining each BTx623 genomic location hit by sequences from both parental lines and then comparing the two parental sequences in a pairwise fashion (P. Klein, personal communication). Polymorphisms identified in this way were classified as DG markers and were used as input in another perl script to score the corresponding DG sequence alleles in each progeny line (P. Klein, personal communication). The resulting data were used for genetic map construction as detailed below.

Genetic Map Construction and Analysis

DG markers were initially ordered based on their physical position along each sorghum chromosome as determined from BLASTN analysis. Subsequently these markers were analyzed and then ordered to construct a genetic map using the MapMaker/EXP V3.0 program (Lander et al., 1987). For a given chromosome, marker genotypic data was entered into a text file, converted to a RAW file, and then prepared for analysis in MapMaker/EXP using the "prepare data" command. Subsequently, the "sequence all" command was used to specify all markers and then the "order" command at LOD 3.0 was used to arrange the markers in order. Markers not placed at LOD 3.0 were re-run using the "order" command to place them at LOD 2.0. Any markers not placed at LOD 2.0 were discarded from further analysis. The robustness of marker order

for a given linkage group was tested using the "ripple" command at LOD 2.0 in which marker order was tested stepwise in groups of four markers. Orders passing the ripple test at LOD 2.0 and greater were subjected to the "map" command to calculate map distances between the markers (Appendix A). The Kosambi mapping function was used to transform recombination frequency into cM distances (Kosambi, 1944). A visual representation of the genetic map was generated using MapChart V2.1 (Voorrips, 2002). Segregation distortion along the linkage groups was calculated using Ant Map V2.0 (Iwata and Ninomiya, 2004).

Results

Approximately 3.1 million useful sequences containing the *NgoM*IV partial restriction site plus the correct four base identification tag at the 5' end were generated from four lanes of DNA run on the Illumina Genome Analyzer II. These 72 bp sequences were clustered and then compared to the genome sequence to identify 4308 markers polymorphic between BTx642 and RTx7000 and uniquely mapped to the sorghum genome. 1534 of these markers contained no more than 15% missing data across the population and were used for subsequent analysis. Within this set of 1534 markers, each RIL was represented by ~8900-60,800 useful sequences. Across the genome, each marker was represented by 357-7014 sequences. On a per RIL basis, each DG marker was sequenced 0-152 times, with an average of ~9 times.

Of the 1534 markers, no recombination was found between approximately one-third of them, leading to a total of 566 markers used in map construction (Appendix A). The map spanned 1130 cM and approximately 583 Mbp, with a total genome coverage

of 87.6% (Table 3.1, Fig. 3.1). Each linkage group contained on average 57 markers, with a genome-wide marker density of 2.0 cM per marker (~1.1 Kbp per marker) and an average of ~526 Kbp per cM (Table 3.1).

Genome-wide, 50.0% of the alleles were homozygous RTx7000, 48.9% were homozygous BTx642, and the remaining 1.1% of the alleles were undetermined (composed of heterozygous regions and missing data). Therefore on a global scale the population segregated close to the expected Mendelian 1:1 segregation ratio. On a local scale, however, deviation from the expected Mendelian 1:1 segregation ratio was seen on six of the ten linkage groups (Table 3.1, Appendix A). The most severe segregation distortion occurred on LG-03 (26%) and LG-10 (53%), in agreement with published results (Xu et al., 2000; Harris, 2007) and a DG-based *Fse*I map (Table 3.2) (B.Weers et al., unpublished). LG-03 had a 21 cM block favoring BTx642 alleles from marker DG178 to marker DG190 and LG-10 had a 34 cM block favoring the RTx7000 allele from marker DG530 to marker DGA550. Error rates were checked by duplicate independent analysis of four of the RILs and BTx642. No errors were detected between duplicates in loci where alleles were sequenced at least four times.

Table 3.1. BTx642 x RTx7000 RIL population *NgoM* IV genetic map marker composition, segregation, density, and coverage.

LG[†]	Marker #	Seg. Distort.[‡]		Length (cM)	Ave. Density (cM/marker)	Length (Kbp)	Ave. Density (Kbp/marker)	Average Kbp/cM	Length (Kbp)[§]	Marker Coverage[¶]
		No.	%							
1	89	1	1.1%	190.4	2.1	72,938.5	819.5	383.1	73,840.6	98.8%
2	55	8	14.5%	125.6	2.3	73,082.6	1,328.8	581.9	77,932.6	93.8%
3	91	24	26.4%	140.2	1.5	73,658.6	809.4	525.4	74,441.2	98.9%
4	80	4	5.0%	143.8	1.8	67,496.1	843.7	469.4	68,034.3	99.2%
5	51	1	2.0%	114.3	2.2	62,159.0	1,218.8	543.8	62,352.3	99.7%
6	59	0	0.0%	121.1	2.1	61,702.2	1,045.8	509.5	62,208.8	99.2%
7	31	0	0.0%	55.2	1.8	52,727.2	1,700.9	955.2	64,342.0	81.9%
8	31	0	0.0%	85.1	2.7	54,362.6	1,753.6	638.8	55,460.3	98.0%
9	20	0	0.0%	35.9	1.8	5,461.7	273.1	152.1	59,635.6	9.2%
10	59	31	52.5%	118.8	2.0	59,063.1	1,001.1	497.2	60,981.6	96.9%
Total	566	69	--	1130.4	--	582,651.5	--	--	659,229.4	--
Average	57	7	10.2%	113.0	2.0	58,265.2	1,079.5	525.6	65,922.9	87.6%

[†]Linkage group.

[‡]Number and percentage of markers showing segregation distortion (P<0.05).

[§]Phytozome: http://www.phytozome.net.

[¶]Current study chromosome length relative to Sorghum bicolor chromosome length from http://www.phytozome.net.

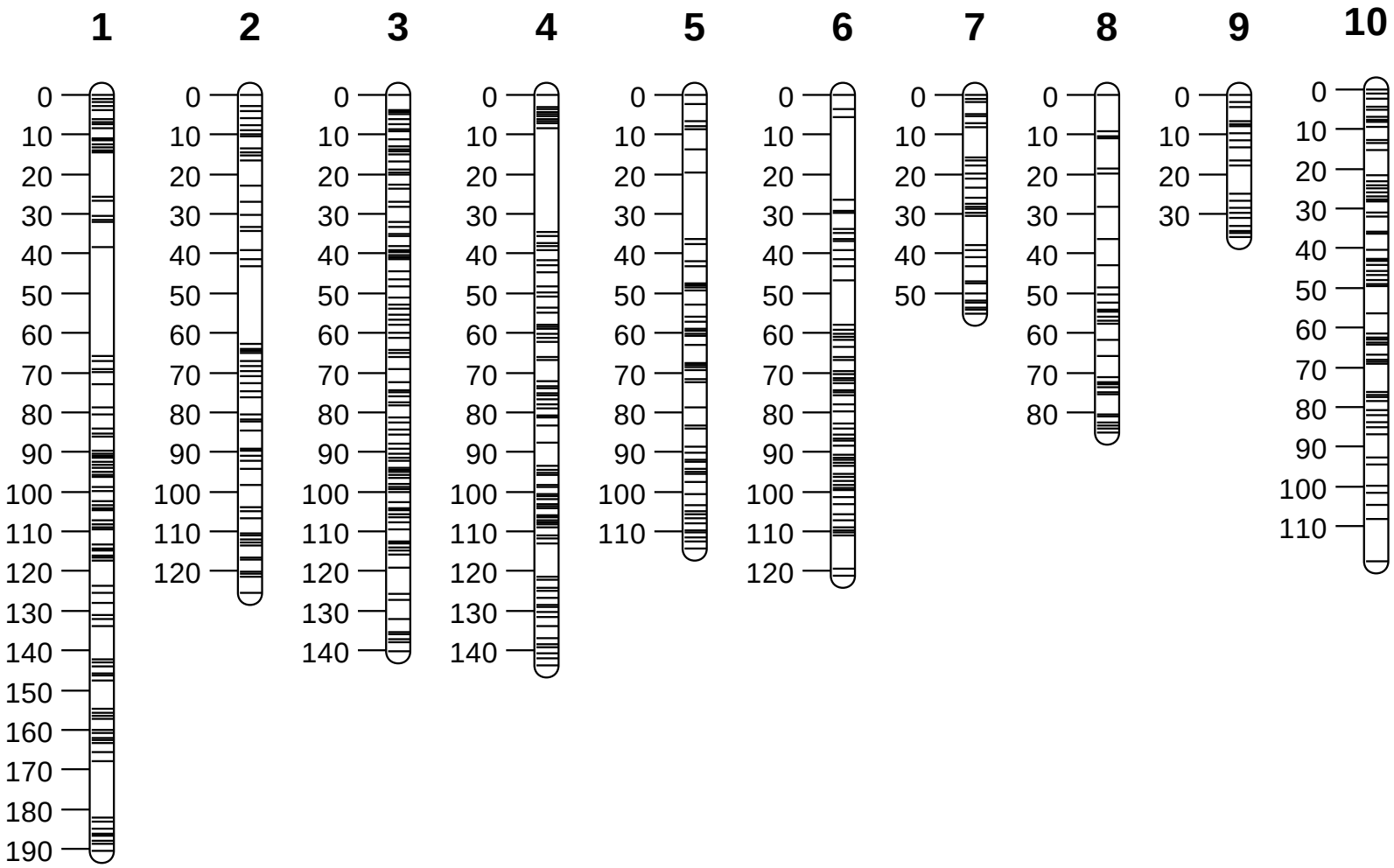


Figure 3.1. BTx642 x RTx7000 RIL population genetic map. Markers are shown as horizontal hashes. Relative positions can be inferred from the centimorgan scale presented on the left of each linkage group. A complete listing of markers is shown in Appendix A.

Table 3.2. BTx642 x RTx7000 RIL population *Fse*I genetic map marker composition, segregation, density, and coverage.

LG[†]	Marker Type (#)[‡]			Seg. Distort.[§]		Length	Ave. Density	Length	Ave. Density	Average	Length	Marker
	DG	SSR	AFLP	No.	%	(cM)	(cM/marker)	(Kbp)	(Kbp/marker)	Kbp/cM	(Kbp)[¶]	Coverage[#]
1	54	4	15	3	4.1%	192.0	2.6	73,394.3	1,005.4	382.3	73,840.6	99.4%
2	39	1	7	10	21.3%	170.0	3.6	77,206.5	1,642.7	454.2	77,932.6	99.1%
3	53	9	10	26	36.1%	130.8	1.8	73,909.0	1,026.5	565.1	74,441.2	99.3%
4	48	4	8	6	10.0%	143.2	2.4	66,979.5	1,116.3	467.7	68,034.3	98.4%
5	27	2	9	5	13.2%	111.1	2.9	62,145.1	1,635.4	559.4	62,352.3	99.7%
6	42	2	6	3	6.0%	108.7	2.2	61,904.4	1,238.1	569.5	62,208.8	99.5%
7	18	4	6	1	3.6%	65.7	2.3	53,119.4	1,897.1	808.5	64,342.0	82.6%
8	20	3	8	3	9.7%	88.0	2.8	55,008.7	1,774.5	625.1	55,460.3	99.2%
9	10	4	2	1	6.3%	36.8	2.3	5,248.3	328.0	142.6	59,635.6	8.8%
10	48	0	6	31	57.4%	121.1	2.2	60,569.2	1,121.7	500.2	60,981.6	99.3%
Total	359	33	77	89	--	1167.4	--	589,484.3	--	--	659,229.4	--
Average	36	3	8	9	16.8%	116.74	2.5	58,948.4	1,278.6	507.4	65,922.9	88.5%

[†]Linkage group.

[‡]DG: digital genotype marker; SSR: simple sequence repeat; AFLP: amplified fragment length polymorphism.

[§]Number and percentage of markers showing segregation distortion ($P<0.05$).

[¶]Phytozome: http://www.phytozome.net.

[#]Current study chromosome length relative to Sorghum bicolor chromosome length from http://www.phytozome.net.

Stay green loci were mapped onto the DG genetic map using trait data that had been previously collected on the BTx642 x RTx7000 RIL population in three locations in Texas over five years: Lubbock, Halfway, and Chillicothe, in 1992-1994 (Xu et al., 2000). Stay green QTL mapped to approximately the same chromosomal regions in this study as were originally identified in the Xu et al. (2000) study (Fig. 3.2). Specific QTL information is shown in Table 3.3.

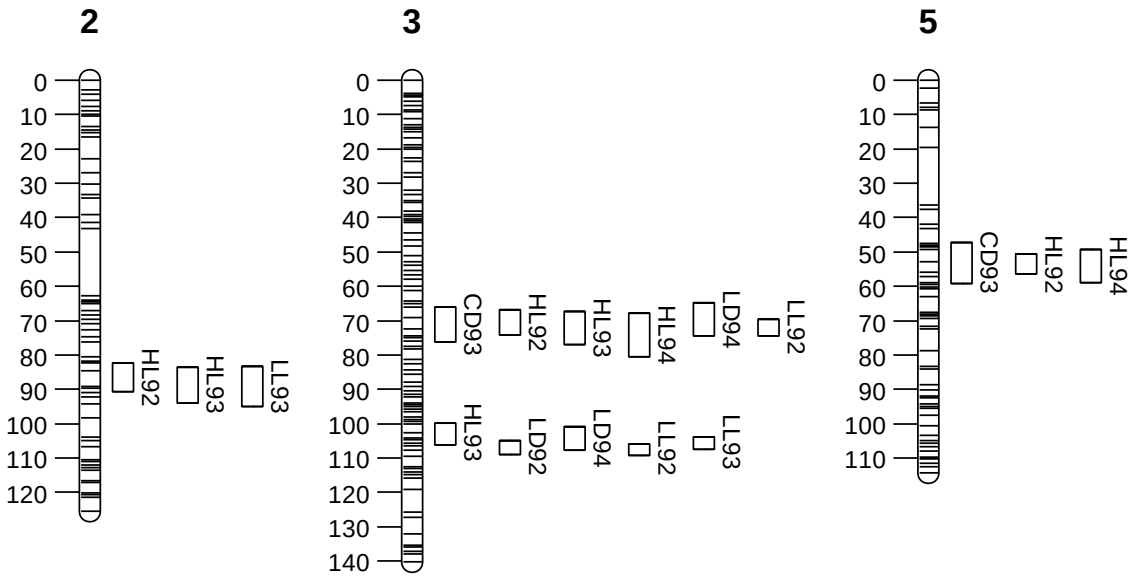


Figure 3.2. Location of stay green loci in the BTx642 x RTx7000 RIL population. Trait data from Chilicothe (C), Halfway (H), and Lubbock , TX (L) grown under limited irrigation (L) or dryland conditions (D) in 1992-1994 (Xu, 2000) was mapped on to the current map. Unfilled bars indicate QTL in which BTx642 increased the magnitude of the trait. Markers are shown as horizontal hashes. Relative positions can be inferred from the centimorgan scale presented on the left of each linkage group. Specific QTL information is shown in Table 3.3.

Table 3.3. Stay green QTL for BTx642 x RTx7000 RILs grown in Chilicothe, Halfway, and Lubbock, TX under limited irrigation and dryland conditions from 1992-1994 by Xu et al., 2000.

Stg[†]	Location	Year	Irrigation	LG[‡]	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
1	Halfway	1993	Limited	3	101.2	4.86	99.7 - 106.1	98.5 - 107.7	-0.34	0.15	3.16
	Lubbock	1992	Limited	3	107.7	4.32	105.8 - 109.3	104.7 - 111.8	-0.20	0.14	3.04
		1992	Dryland	3	106.6	5.26	105.0 - 109.0	102.7 - 110.8	-0.14	0.18	3.15
		1993	Limited	3	105.7	6.61	104.0 - 107.5	103.2 - 111.8	-0.25	0.20	3.05
		1994	Dryland	3	106.5	5.16	100.9 - 107.7	99.6 - 109.2	-0.27	0.14	3.15
2	Chilicothe	1993	Dryland	3	71.3	4.29	66.1 - 76.1	66.0 - 80.9	-0.25	0.11	3.13
	Halfway	1992	Limited	3	70.4	4.68	66.8 - 74.2	62.0 - 81.3	-0.25	0.14	2.92
		1993	Limited	3	72.4	2.66	67.4 - 76.9	66.0 - 82.4	-0.25	0.07	3.16
		1994	Limited	3	71.2	4.46	67.9 - 80.4	66.2 - 81.4	-0.33	0.15	3.18
	Lubbock	1992	Limited	3	72.5	3.10	69.6 - 74.3	66.1 - 76.2	-0.17	0.10	3.04
		1994	Dryland	3	69.0	2.88	64.7 - 74.3	66.2 - 77.2	-0.21	0.08	3.15
3	Halfway	1992	Limited	2	84.8	3.14	82.4 - 90.8	77.9 - 96.4	-0.19	0.08	2.92
		1993	Limited	2	91.1	4.45	83.5 - 94.0	76.2 - 103.4	-0.32	0.13	3.16
	Lubbock	1993	Limited	2	91.1	2.43	83.3 - 95.0	80.2 - 101.3	-0.14	0.06	3.05
4	Chilicothe	1993	Dryland	5	55.9	3.03	47.3 - 59.1	42.9 - 60.9	-0.21	0.08	3.13
	Halfway	1992	Limited	5	53.0	2.89	50.6 - 56.4	49.2 - 62.9	-0.18	0.08	2.92
		1994	Limited	5	55.7	2.40	49.4 - 59.0	43.0 - 67.5	-0.23	0.07	3.18

[†]Stay green locus.

[‡]Linkage group.

[§]QTL peak in cM.

[¶]QTL width in cM at either 1 (LOD-1) or 2 (LOD-2) LOD units below the peak.

[#]Additive effect (visual rating 1-5 where 1 corresponds to little leaf senescence and 5 corresponds to total leaf senscence at grain matuirty). Sign is with respect to RTx7000.

[††]Proportion of total phenotypic variance explained by the QTL.

Discussion

An 1130 cM genetic map based on a BTx642/RTx7000 RIL population consisting of 90 F_{12} RILs was generated using digital genotyping technology developed to utilize the high throughput sequencing capability of the Illumina Genome Analyzer II. The DG genetic map had increased resolution compared with the prior genetic map based primarily on RFLP and SSR markers described by Xu et al. (2000). DG markers

were generated using a methylation-sensitive restriction enzyme, which facilitated marker discovery in the euchromatic regions of the sorghum genome that have relatively high rates of recombination. The DG markers mapped to unique and specific positions on the sorghum genome sequence. While genetic maps are often based on recombination frequency, the DG markers allow combined analysis of recombination frequency and physical location/distances between DG markers. Therefore QTL can be located on the genetic and physical maps of the sorghum genome facilitating the subsequent discovery of genes that modulate target traits. Additionally, the current map improves the DG-based *Fse*I map (B. Weers et al., unpublished) in that *Fse*I has an 8-bp recognition site that enables marker discovery at lower frequency (per bp) compared to *NgoM*IV, an enzyme that recognizes a nested 6-bp sequence. Both enzymes are methylation-sensitive, enhancing marker discovery in euchromatic gene-rich regions of the genome. Further, the current map contains 103 more markers than the *Fse*I map, in effect decreasing marker density from 2.5 cM/marker (~1.3 Kbp/marker) to 2.0 cM/marker (~1.1 Kbp/marker), thereby potentially increasing QTL resolution.

The BTx642/RTx7000 DG map spans most of the sorghum genome with the exception of chromosome 9 where marker coverage spanned only 9.2% of this chromosome. The low marker coverage on this chromosome and a large region on LG-06 that encodes the major sorghum maturity gene, *ma1*, may be due to the presence of DNA in BTx642 that is similar in terms of diversity to DNA in RTx7000. BTx642 was derived from IS12555 through the Sorghum Conversion Program by introgression of dwarfing and early flowering regions of the genome from BTx406 (i.e. *ma1*). Both

RTx7000 and BTx406 were derived from milo (durra) and kafir sorghums introduced into the U.S. in the period from 1875-1925, so it is possible that portions of chromosome 9 in the parental lines are of common descent, making it difficult or impossible to detect polymorphisms between the parents in this region of the genome. In addition, a dwarfing locus (putative *Dw1*) was found on chromosome 9 by Brown et al. (2008). Therefore this region of the genome may have been a target of selection by early breeders in the conversion program.

The four stay green loci previously mapped by Xu et al. (2000) were located on the DG-genetic map created during this study using phenotypic data collected by Xu et al. (2000). Xu used a visual rating scale from one to five to phenotype the stay-green trait in the RIL population, with a rating of one corresponding to little leaf senescence and five corresponding to total leaf senescence at grain maturity. The stay green QTL were located in approximately the same locations on LG-02 (*Stg3*), LG-03 (*Stg1*, *Stg2*) and LG-05 (*Stg4*) as previously reported (Xu et al., 2000) and each QTL interval was smaller due to more markers in the respective regions. This information provides the basis for relating the *Stg* QTL to QTL that modulate a wide range of other traits analyzed in this study.

CHAPTER IV

ENVIRONMENTAL CONDITIONS AND TRAIT ANALYSES

Introduction

The materials and methods contained herein pertain to those used in subsequent chapters. Materials and methods are described for experiments involving the environmental conditions and collection and analysis of data for BTx642, RTx7000, and a RIL population derived from these two genotypes. Traits including days to flower and stalk length, and leaf size, shoot biomass, and root size parameters for plants grown to 52 DAE or to anthesis under field conditions in 2008-2010 and greenhouse conditions were measured. Trait analyses including analysis of variance, broad sense heritability, and QTL mapping were conducted.

Materials and Methods

Greenhouse at Anthesis

On 1/14/11 ten seeds each of the BTx642 x RTx7000 RIL population and parental lines were planted directly into 5 gallon pots, two pots per line, containing a 2:1 mix of coarse vermiculite (Sun Gro Horticulture) to Brazos County, TX silty loam soil. Additionally, 20 g of slow-release fertilizer osmocote 14-14-14 (Scotts-Sierra Horticultural Products) was added at a rate of 20 g per pot at the time of planting and every 30 days thereafter. The plants were grown under well-watered conditions in the greenhouse under 10 hour days. The average daily minimum temperature for the experiment was 22.4° C and the average daily maximum temperature was 37.9° C. Emergence occurred on 1/18/11. At 16 DAE, the plants were thinned to three plants per

pot. The plants were harvested as each RIL or parental genotype reached anthesis, which ranged from 46-79 DAE. Three plants of each RIL and parental line from each of two reps were harvested. Leaf size parameters (length, width, and area) were measured with a LI-3100C Area Meter (LI-COR Biosciences). Other traits measured included flowering time, stalk length, total leaf area, and fresh weight of the shoot, stalk, stem, leaf, leaf sheath, peduncle, and panicle. All tissues were dried at 71° C for three days to assess dry weights.

Field 2008 at Anthesis

Each member of the BTx642 x RTx7000 RIL population and parental line was planted in Norwood silty clay loam soil (fine-silty, mixed (calcareous), thermic Typic Udifluvent) in duplicate in a randomized block design at the Texas A&M Research Farm located near Snook, Texas on 4/1/08. The blocks were arrayed in 20 rows 4.6 m long and spaced 76 cm apart. Two buffer rows were planted on each end of the block. Each block was offset from the next block by approximately 1.5 m. The plants emerged on 4/6/08 and were thinned to a within-row spacing of 10 cm at 16 DAE. The average daily maximum temperature was 31.7º C and the average daily minimum temperature was 20.0º C. The population received 19.6 cm of natural rainfall during the growing season. Flood irrigation was used to supplement natural rainfall, approximately weekly after emergence. The population was harvested on 6/19/08 (74 DAE), approximately at anthesis for the population. Three plants of each RIL and parental line from each of two reps were harvested. Leaf size parameters (length, width, and area) were measured with a LI-3100C Area Meter (LI-COR Biosciences). Total leaf area and flowering time were

also measured at anthesis. At grain maturity (approximately 7/27/08) panicles were harvested. Panicle fresh weight was measured. Panicles were air-dried to assess dry weights.

Field 2009 at Anthesis

Each member of the BTx642 x RTx7000 RIL population and parental line was planted in Norwood silty clay loam soil (fine-silty, mixed (calcareous), thermic Typic Udifluvent) in duplicate in a randomized block design at the Texas A&M Research Farm located near Snook, Texas on 4/3/09. The blocks were arrayed in 20 rows 4.6 m long and spaced 76 cm apart. Two buffer rows were planted on each end of the block. Each block was offset from the next block by approximately 1.5 m. The plants emerged on 4/8/09 and were thinned to a within-row spacing of 10 cm at 16 DAE. The average daily maximum temperature was 33.3º C and the average daily minimum temperature was 21.1º C. The population received 24.9 cm of natural rainfall during the growing season. Flood irrigation was used to supplement natural rainfall, approximately weekly after emergence. The population was harvested on 6/23/09 (76 DAE), approximately at anthesis for the population. Three plants of each RIL and parental line from each of two reps were harvested. Leaf size parameters (length, width, and area) were measured with a LI-3100C Area Meter (LI-COR Biosciences). Other traits measured included flowering time, total leaf area, stalk length, shoot weight, stalk weight, leaf total weight, and panicle weight. Panicles were harvested at grain maturity, approximately 8/8/09. Panicle weight was measured. All tissues were dried at 71° C for three days to assess dry weights.

Field 2009 at 52 DAE

Each member of the BTx642 x RTx7000 RIL population and parental line was planted in Norwood silty clay loam soil (fine-silty, mixed (calcareous), thermic Typic Udifluvent) in duplicate in a randomized block design at the Texas A&M Research Farm located near Snook, Texas on 4/3/09. The blocks were arrayed in 20 rows 4.6 m long and spaced 76 cm apart. Two buffer rows were planted on each end of the block. Each block was offset from the next block by approximately 1.5 m. The plants emerged on 4/8/09 and were thinned to a within-row spacing of 10 cm at 16 DAE. The average daily maximum temperature was 28.3º C and the average daily minimum temperature was 17.2º C. The population received 18.7 cm of natural rainfall during the growing season. Flood irrigation was used to supplement natural rainfall, approximately weekly after emergence. The population was harvested on 5/30/09 (52 DAE), during growth stage two. Three plants of each RIL and parental line from each of two reps were harvested. Root, shoot, and total plant fresh and dry weights were measured. Prior to drying, roots were scanned with an Epson dual lens scanner model V700 at 400 dpi. Scanned images were analyzed with WinRhizo V.2008a software (Regent Instruments), which determined root length at various root diameters, total root length, root surface area at various root diameters, total root surface area, root volume at various root diameters, total root volume, number of root tips at various root diameters, total root tip number, total number of root forks, and average root diameter.

Field 2010 at Anthesis

Each member of the BTx642 x RTx7000 RIL population and parental line was planted in Norwood silty clay loam soil (fine-silty, mixed (calcareous), thermic Typic Udifluvent) in duplicate in a randomized block design at the Texas A&M Research Farm located near Snook, Texas on 3/31/10. The blocks were arrayed in 20 rows 4.6 m long and spaced 76 cm apart. Two buffer rows were planted on each end of the block. Each block was offset from the next block by approximately 1.5 m. The plants emerged on 4/6/09 and were thinned to a within-row spacing of 10 cm at 16 DAE. The average daily maximum temperature was 31.1º C and the average daily minimum temperature was 20.6º C. The population received 33.2 cm of natural rainfall during the growing season. The population was harvested as the RILs reached anthesis. The earliest RILs reached anthesis by 6/7/10 and the latest RILs reached anthesis by 6/23/10. Five plants of each RIL and parental line from each of two reps were harvested. Leaf size parameters (length, width, and area) were measured with a LI-3100C Area Meter (LI-COR Biosciences). Other traits measured included flowering time, total leaf area, stalk length, and fresh weights of the shoot, stalk, stem, leaf, leaf sheath, peduncle, and panicle. Panicles were harvested at grain maturity, approximately 8/1/10. Panicle fresh weight was measured. All tissues were dried to assess dry weights.

Field 2010 at 52 DAE

Each member of the BTx642 x RTx7000 RIL population and parental line was planted in Norwood silty clay loam soil (fine-silty, mixed (calcareous), thermic Typic Udifluvent) in duplicate in a randomized block design at the Texas A&M Research Farm

located near Snook, Texas on 3/31/10. The blocks were arrayed in 20 rows 4.6 m long and spaced 76 cm apart. Two buffer rows were planted on each end of the block. Each block was offset from the next block by approximately 1.5 m. The plants emerged on 4/6/09 and were thinned to a within-row spacing of 10 cm at 16 DAE. The average daily maximum temperature was 28.9º C and the average daily minimum temperature was 17.2º C. The population received 7.9 cm of natural rainfall during the growing season. The population was harvested as the RILs reached anthesis. Flood irrigation was used to supplement natural rainfall, approximately weekly after emergence. The population was harvested on 5/28/10 (52 DAE), during growth stage two. Three plants of each RIL and parental line from each of two reps were harvested. Root, shoot, and total plant fresh and dry weights were measured. Prior to drying, roots were scanned with an Epson dual lens scanner model 10000XL at 600 dpi. Scanned images were analyzed with WinRhizo V.2008a software (Regent Instruments), which determined root length at various root diameters, total root length, root surface area at various root diameters, total root surface area, root volume at various root diameters, total root volume, number of root tips at various root diameters, total root tip number, total number of root forks, and average root diameter.

Trait Analyses[1]

Analysis of Variance

Trait data for each RIL were averaged and then tested for mean differences across the population using a one-way analysis of variance (ANOVA) in SPSS 16.0 for Windows (Brannan et al., 2007) at the 95% confidence level. The null hypothesis that all means were equal was rejected in favor of the alternate hypothesis that all means were not equal any time the calculated F value exceeded the table value at an alpha value equal to 0.05 with the appropriate degrees of freedom. Traits with insignificant mean differences were discarded from further analysis.

Remaining traits were further analyzed with ANOVA using SAS V9.2 (SAS Institute Inc., 2008) to examine covariates and to calculate broad sense heritability. Covariates and broad sense heritability were examined both across years in the field and across field and greenhouse environments. Trait data were entered into SAS and analyzed using the MIXED PROC with all effects random.

For illustration, a sample data analysis under field conditions in 2008, 2009, and 2010 is shown. An example script used in the SAS editor window is presented in Figure 4.1. Note that trait data starts on line six and has been truncated for brevity. The SAS output is shown in Table 4.1, in which statistics for the covariates rep, entry, year, year*entry, and residuals are shown. SAS computed values for the covariates (Table 4.2) by using the mean square values and the expected mean square equations.

[1]Methods herein were reviewed by Stanley Luck, Ph.D., a statistician and QTL expert from Dupont, Wilmington, DE, and Petra Wolters, Ph.D., a plant geneticist, from Dupont, Wilmington, DE.

In the current example (Table 4.1), the covariate for year*entry was computed by substituting the Var(Residual) value (3.778) into the expected mean square equation: 10.574 = Var(Residual) + 1.996*Var(year*entry). Similar operations were conducted to calculate the remaining covariates. The significance level for a given covariate is indicated in the column titled "Pr > F" in Table 4.1.

```
ods listing close;
ods rtf file='C:\Ftyear';
data RIL;
input entry rep year FT;
cards;
1        1        1        70
2        1        1        70
3        1        1        74
4        1        1        73
5        1        1        74
6        1        1        70
7        1        1        74
8        1        1        75
9        1        1        74
10       1        1        78
11       1        1        82
12       1        1        70
13       1        1        75
14       1        1        71
15       1        1        78
16       1        1        78
17       1        1        74
18       1        1        76
19       1        1        82
20       1        1        80
;
proc mixed method=type3;
class rep year entry;
model FT = /solution;
random rep entry|year/solution;
run;
ods rtf close;
ods listing;
```

Figure 4.1. Analysis of variance SAS script.

Table 4.1 SAS analysis of variance.

Type 3 Analysis of Variance								
Source	**DF**	**Sum of Squares**	**Mean Square**	**Expected Mean Square**	**Error Term**	**Error DF**	**F Value**	**Pr > F**
rep	1	1.766544	1.766544	Var(Residual) + 272 Var(rep)	MS(Residual)	271	0.47	0.4947
entry	90	7679.824307	85.331381	Var(Residual) + 1.9945 Var (year*entry) + 5.9834 Var(entry)	0.9994 MS(year*entry) + 0.0006 MS(Residual)	180.07	8.07	<.0001
year	2	8908.719767	4454.359884	Var(Residual) + 1.9927 Var (year*entry) + 181.34 Var(year)	0.9986 MS(year*entry) + 0.0014 MS(Residual)	180.19	421.65	<.0001
year*entry	180	1903.287481	10.573819	Var(Residual) + 1.9956 Var (year*entry)	MS(Residual)	271	2.80	<.0001
Residual	271	1023.733456	3.777614	Var(Residual)	.	.	.	.

Table 4.2. SAS analysis of variance covariate estimates.

Covariance Parameter Estimates	
Cov Parm	**Estimate**
rep	0.00739
entry	12.4947
year	24.5059
year*entry	3.4056
Residual	3.7776

Broad sense heritability was calculated using the equation (S. Murray, personal communication):

$$h_b^2 = H = \frac{\hat{\sigma}_G^2}{\hat{\sigma}_G^2 + \frac{\hat{\sigma}_{GxE}^2}{e} + \frac{\hat{\sigma}_{error}^2}{re}}$$

In this equation genotypic variation ("entry") is shown as $\hat{\sigma}_G^2$, genetic x environment variation ("year*entry") is $\hat{\sigma}_{GxE}^2$, and error variation ("residual") is $\hat{\sigma}_{error}^2$. The number of reps is indicated as "*r*," and "*e*" indicates the number of environments.

Composite Interval Mapping (CIM) QTL Analysis

For a given trait, data from each of the 90 members of the BTx642 x RTx7000 RIL population were averaged by RIL across reps and then subjected to QTL analysis with WinQTL Cartographer V2.5 (Wang et al., 2007). A mapping file was constructed from genotypic data (discussed in Chapter III) and phenotypic data. The mapping file

was constructed from four component files. File 1 contained a listing of the number of linkage groups and the number of markers per linkage group (Fig. 4.2). File 2 contained a listing of the order of markers, with each line representing one linkage group and position zero on a given linkage group defined as the first marker listed (Fig. 4.3). File 3 contained the genetic position in centimorgans for each marker listed in File 2. As in File 2, individual linkage groups were represented as single lines with the first position on each linkage group designated as zero centimorgans (Fig. 4.4). In File 4 genotypic data for each locus in every RIL as well as phenotypic data for each RIL were arrayed (Fig. 4.5). Rows corresponded to individual RILs and columns corresponded to individual markers. Homozygous loci from RTx7000 were coded as "2" and homozygous loci from BTx642 were coded as "0." Heterozygous loci and loci with missing data were coded as "*". Phenotypic data for each RIL were arrayed in columns adjacent to genotypic data for the last marker on linkage group 10 (data not shown in Fig. 4.5). Trait labels were arrayed in order adjacent to "Genotypes" in the first line of File 4.

LG-01	73
LG-02	47
LG-03	72
LG-04	60
LG-05	38
LG-06	50
LG-07	28
LG-08	31
LG-09	16
LG-10	54

Figure 4.2. File 1: Number of markers per linkage group.

DGA1	DGA2	DGA4	DGA7	DGA8	txa74	txa3050	DGA10	DGA11	DGA12	DGA13	DGA14	DGA16
DGA75	DGA76	DGA77	DGA78	DGA80	DGA82	txa904	DGA84	DGA85	txa15265	DGA86	DGA88	txa15331
DGA143	DGA145	txa5895	txa261	DGA146	DGA148	DGA149	DGA151	DGA152	DGA153	DGA154	DGA156	DGA157
txp504	DGA238	DGA239	DGA240	DGA242	DGA246	DGA248	txa2669	DGA251	txa2966	txa2676	DGA252	DGA253
DGA310	DGA311	Y12464	DGA312	DGA313	DGA316	txa6019	txa2022	DGA317	DGA318	DGA319	DGA320	txp30
DGA352	DGA353	DGA354	DGA355	DGA359	DGA362	DGA363	DGA366	DGA368	DGA369	DGA370	DGA371	DGA372
DGA418	txp40	txa23	DGA419	txp417	DGA420	DGA421	txa15137	DGA422	DGA425	txa15004	txp481	txa15364
txp273	DGA441	txa2034	txa15351	txa2594	txa15076	cup47	DGA443	DGA444	txa6206	DGA446	DGA447	txa15057
txp289	DGA468	DGA469	txp459	DGA470	DGA471	txp410	DGA473	txa15353	DGA474	DGA475	DGA477	txa15261
DGA481	DGA482	DGA483	DGA484	DGA485	DGA487	DGA489	DGA490	DGA491	DGA493	DGA494	DGA495	DGA496

Figure 4.3. Marker order per linkage group (partial file).

0.0	2.4	3.0	4.7	5.3	6.0	8.9	10.6	11.7	12.8	15.2	16.3	16.9
0.0	1.5	3.3	5.7	7.4	8.0	9.7	10.3	10.9	12.0	15.0	15.6	17.3
0.0	8.6	9.7	12.1	15.1	15.7	16.8	21.0	22.3	23.4	24.5	32.1	32.7
0.0	1.2	3.6	4.8	6.2	7.7	32.8	33.4	35.1	36.2	38.6	39.2	40.3
0.0	2.4	6.8	7.4	8.0	9.7	12.6	13.3	14.4	18.1	22.2	26.3	26.9
0.0	1.7	9.0	9.7	10.3	12.0	12.6	15.0	17.4	20.4	30.5	32.3	33.4
0.0	1.8	3.5	6.5	7.6	8.7	10.4	11.0	15.4	20.0	22.9	24.6	27.6
0.0	2.4	6.1	9.8	10.9	14.6	31.2	32.4	46.6	47.2	48.6	50.7	51.3
0.0	0.6	6.6	10.8	11.4	13.1	13.7	14.3	19.4	25.2	25.8	26.9	29.3
0.0	1.1	1.7	2.3	8.1	9.8	11.6	14.6	15.2	15.8	22.4	23.0	23.6

Figure 4.4. File 3: Marker centimorgan position per linkage group (partial file).

IndLabel	Genotypes	FTK1	FTK2	FTN1	FTN2	FTE1	FTE2	FTI1	FTI2			
IND*01	0	0	0	0	0	0	0	0	0	*	0	0
IND*02	0	0	0	0	0	0	0	0	0	0	2	2
IND*03	0	0	0	0	0	0	0	0	0	0	0	0
IND*04	2	2	2	2	2	2	2	2	2	2	2	2
IND*05	2	2	2	2	2	2	2	2	2	2	2	2
IND*06	2	2	0	0	0	0	0	0	0	0	0	0
IND*07	2	2	2	2	2	2	2	2	2	2	2	2
IND*08	0	0	0	0	0	0	0	0	2	2	2	2
IND*09	2	2	2	2	2	2	2	2	2	*	2	2
IND*10	2	2	2	2	2	2	2	2	2	2	2	2
IND*11	2	2	2	2	2	2	2	2	2	2	2	2
IND*12	0	0	0	0	0	0	0	0	0	0	0	0
IND*13	2	2	2	2	2	2	2	2	2	2	2	2
IND*14	2	2	2	2	*	2	2	2	2	2	2	2
IND*15	0	0	0	0	0	0	0	0	0	0	0	0
IND*16	0	0	0	0	0	0	0	0	0	*	0	0
IND*17	0	0	0	0	0	0	0	0	0	0	0	0
IND*18	2	2	2	2	2	2	2	2	2	0	0	0
IND*19	2	2	2	2	2	2	2	2	2	2	2	2
IND*20	2	0	0	0	0	0	0	0	0	0	0	0
IND*21	2	2	2	0	0	0	0	0	0	0	0	0
IND*22	2	2	2	2	2	2	2	2	2	2	2	2
IND*23	2	2	2	2	2	2	0	0	0	*	0	0
IND*24	2	2	2	2	2	2	2	2	2	2	2	2
IND*25	0	0	0	0	0	0	0	0	0	*	0	0
IND*26	0	0	0	0	0	0	0	0	0	*	0	0
IND*27	2	2	2	2	2	2	2	2	2	2	2	2
IND*28	2	2	2	2	2	2	2	2	2	2	2	2
IND*29	2	2	2	2	2	2	2	2	2	2	2	2
IND*30	2	2	2	2	2	2	2	2	2	2	2	2
IND*31	2	2	2	2	2	2	2	2	2	2	2	0
IND*32	2	2	2	2	2	2	2	2	2	2	2	2
IND*33	0	0	0	0	0	0	0	2	2	2	2	2
IND*34	0	0	0	0	0	0	0	0	0	0	0	0
IND*35	0	0	0	0	0	0	0	0	0	0	0	0
IND*36	0	0	0	0	0	0	0	0	0	0	0	0
IND*37	0	2	2	2	2	2	2	2	2	*	2	2
IND*38	0	0	0	0	0	0	0	0	0	0	0	0
IND*39	2	2	2	*	2	2	2	2	2	*	2	2
IND*40	0	0	0	0	0	0	0	0	2	2	2	2
IND*41	2	2	2	2	2	2	2	2	2	2	2	0
IND*42	2	2	2	2	2	2	2	2	2	2	2	2
IND*43	0	0	*	*	*	*	*	*	0	0	2	*
IND*44	2	2	2	2	2	2	2	2	2	2	2	2
IND*45	0	0	0	0	0	0	0	0	0	0	0	0
IND*46	2	0	0	0	0	0	0	0	0	0	0	0
IND*47	2	2	2	2	2	2	2	2	2	2	2	2
IND*48	0	0	0	0	0	0	0	0	0	0	0	0
IND*49	0	0	0	0	0	*	0	0	0	0	0	0
IND*50	0	0	0	0	0	0	0	0	0	0	0	0

Figure 4.5. File 4: Genotypic data for each RIL at all loci and phenotypic data for each RIL (partial file).

The QTL source mapping file was generated by selecting “New” in the WinQTL Cartographer main menu and then proceeding through six steps. In step 1, basic information including linkage group number (10), trait number (8), other traits (0), sample size (91), symbol for missing traits (.), and cross type (Ri1) were entered as well as information for the marker genotype table: “2” for AA (RTx7000), “0” for aa (BTx642), and “*” for missing and heterozygous loci (Fig. 4.6). In step 2, map information was entered including map function (Kosambi), position type (position), and position unit (centimorgan) (Fig. 4.7). Additionally, the number of linkage groups and the number of markers per linkage group were entered by locating File 1 (discussed previously) via the “Browse” button and then importing the file using the “Send Data” button. Step 3 involved importing marker labels (File 2, discussed previously) and marker positions (File 3, discussed previously). Marker labels and positions were imported by selecting either “Labels” or “Positions” and then importing the respective files in the same manner in which File 1 was imported (Fig. 4.8, Fig. 4.9).

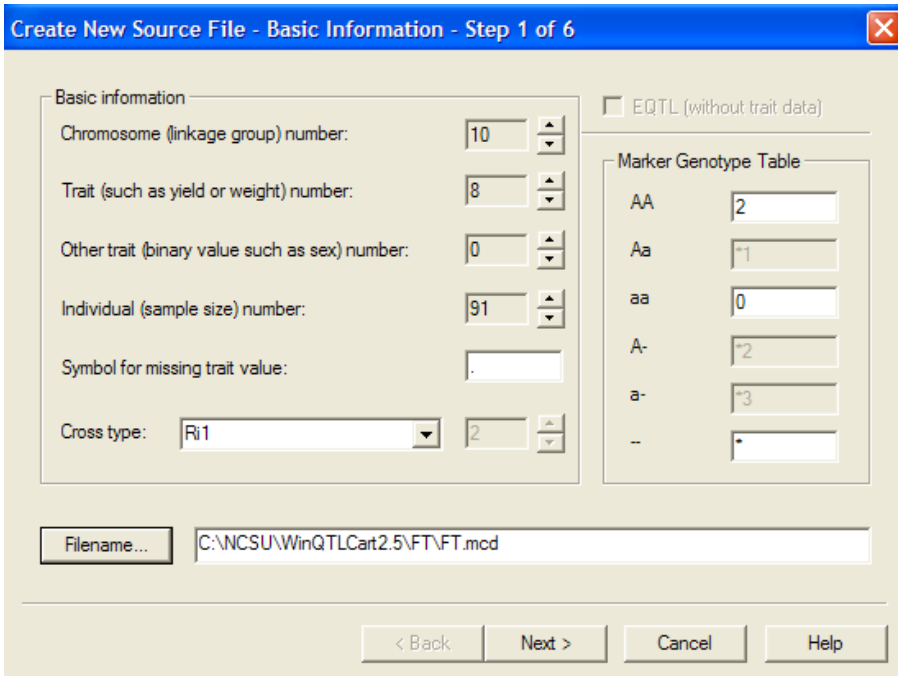


Figure 4.6. WinQTL cartographer source file basic information.

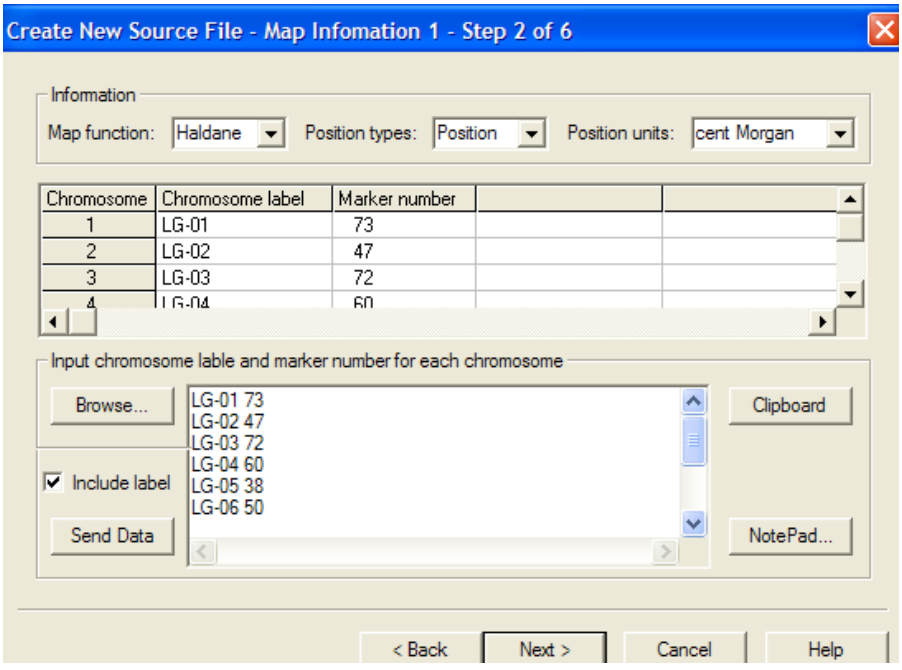


Figure 4.7. Map function, position type, and number of markers per linkage group.

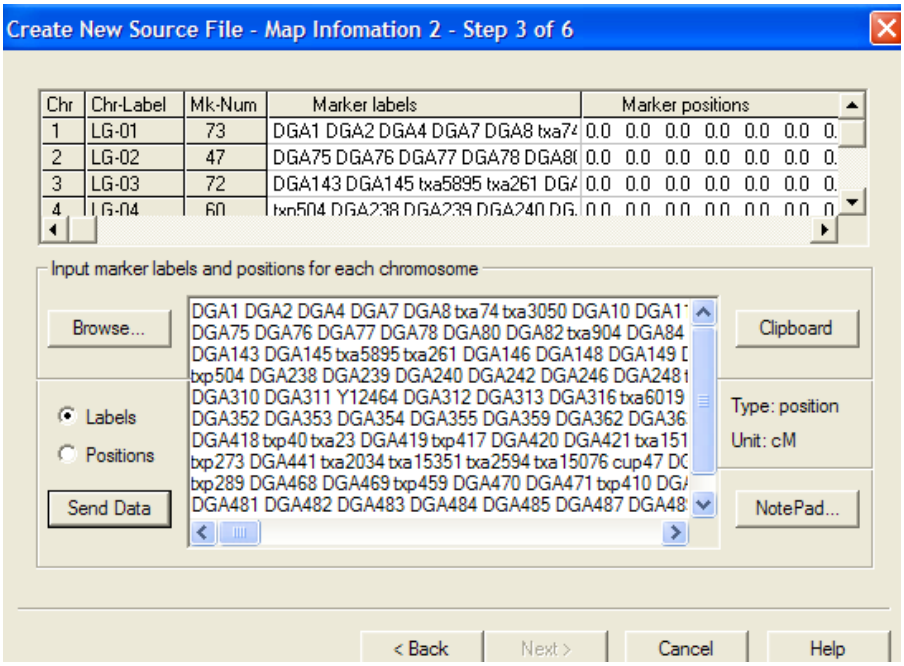


Figure 4.8. Marker labels per linkage group.

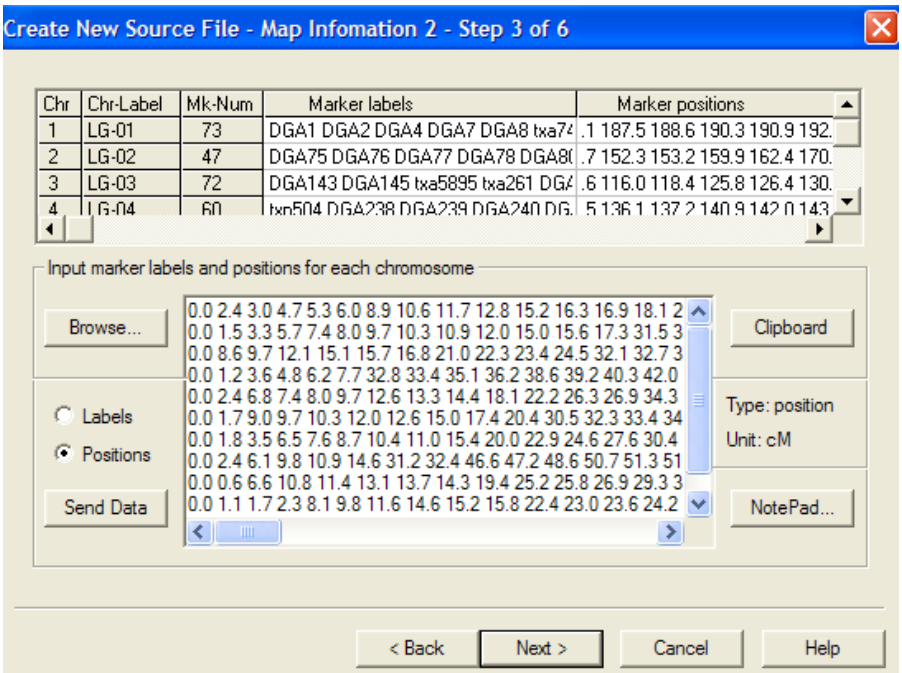


Figure 4.9. Marker positions per linkage group.

Cross information was entered in steps 4 and 5. In step 4, "All data in one file" and "Data format 1" were selected (Fig. 4.10). In step 5 genotypic and phenotypic data were entered by importing File 4 (discussed previously) (Fig. 4.11). In Figure 4.12 step 6 is shown which provides a summary of all genotypic and phenotypic information entered for each RIL. The mapping source file was generated by selecting the "Finish" button. The accuracy of the source file was confirmed by the absence of any "Warning" text files under the text file menu in Figure 4.13.

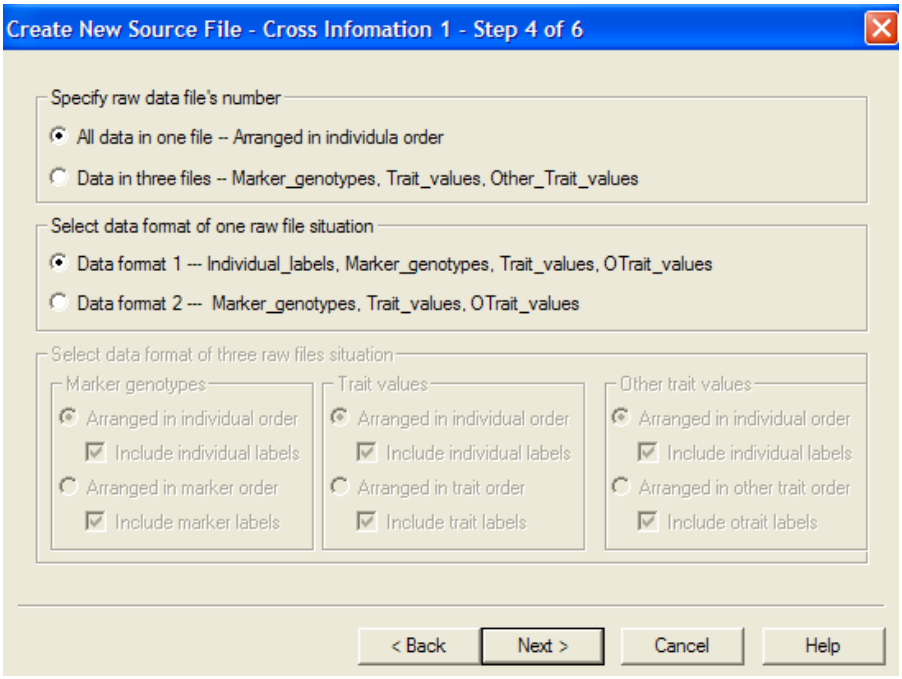


Figure 4.10. Cross information data format.

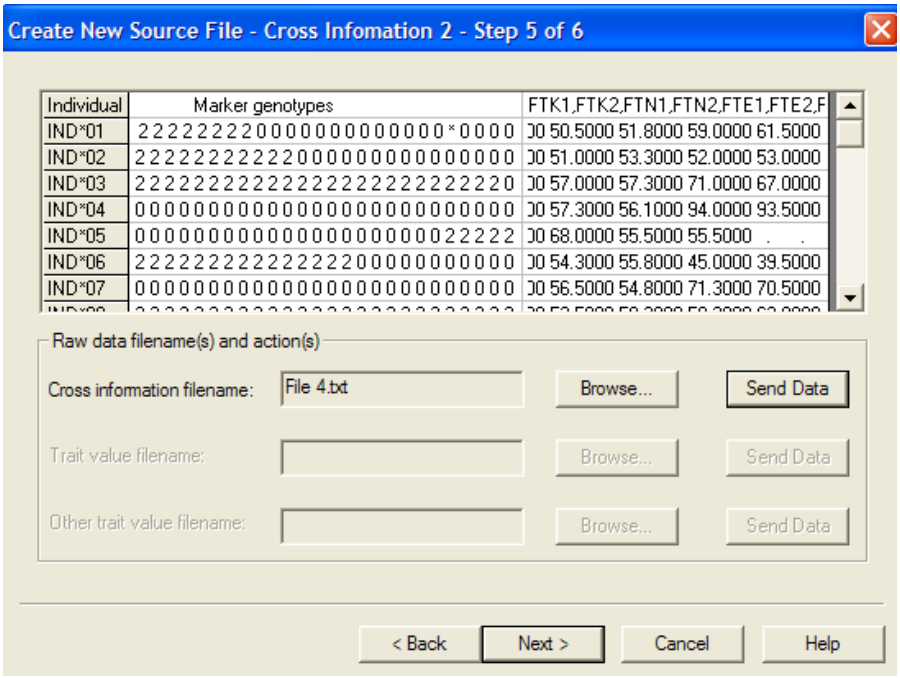


Figure 4.11. Cross information.

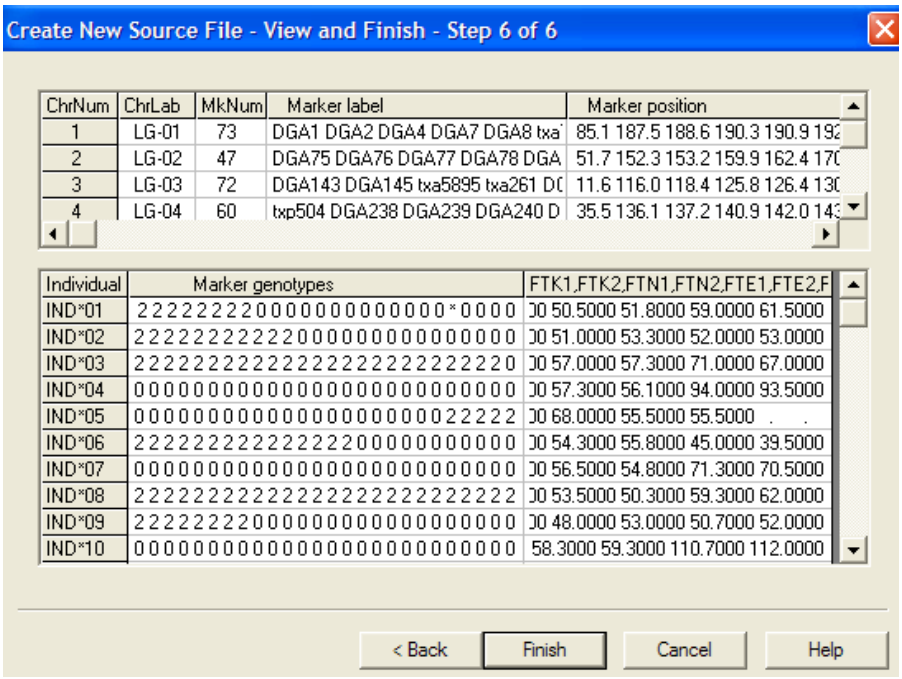


Figure 4.12. Source file setup summary.

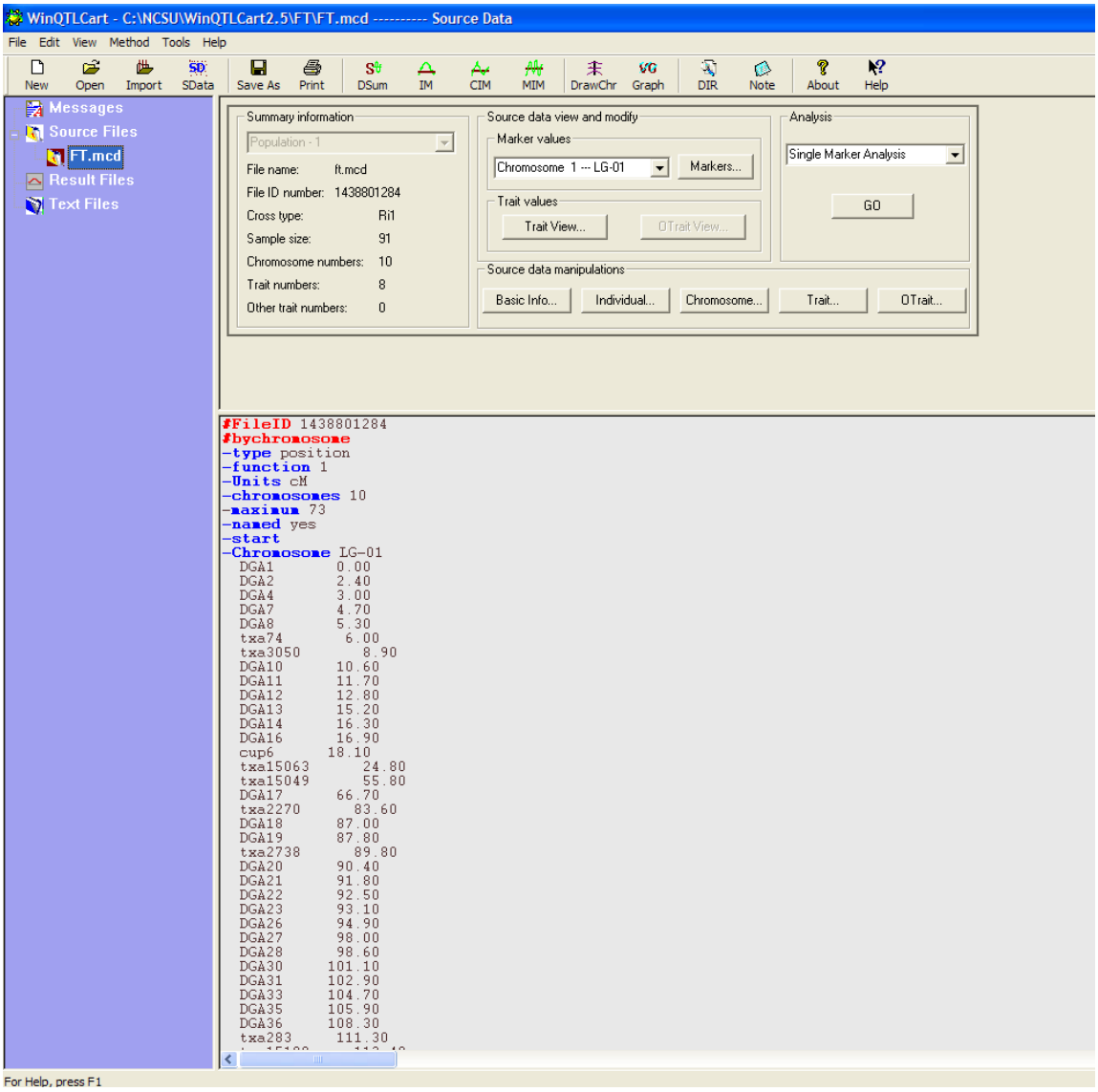


Figure 4.13. Source file successful creation.

Composite interval mapping was conducted by selecting "CIM" in the main WinQTL Cartographer menu. Initially, the likelihood ratio (LR) threshold was set to the manual default value of 11.5 and the walk speed to 1.0 cM (Fig. 4.14). Subsequently the data were permuted 1000 times at a confidence level of 95% to establish a significance threshold (Fig. 4.15). The output of the permutations was ranked in descending order and the 50^{th} rank (95%) was used as the significance level (Table 4.3). All LR scores in Table 4.1 were converted to log of odds (LOD) scores by multiplying by 0.217. Only QTL at or above the calculated LOD threshold for a given trait were reported. The size of a given QTL was determined as the width at one LOD unit below the QTL's peak. The LOD score of a given QTL was defined as the LOD value at the QTL's peak, as determined by using the "Trace Coordinate in Graph" function located in the "Setting" menu and the "Show trace hairs" function in the "Set Display Parameters" menu located in the "Setting" menu (Fig. 4.16). The exact LOD score at the intersection of the trace hairs was reported in the upper right corner of the mapping output window (LOD 5.43 in Fig. 4.16).

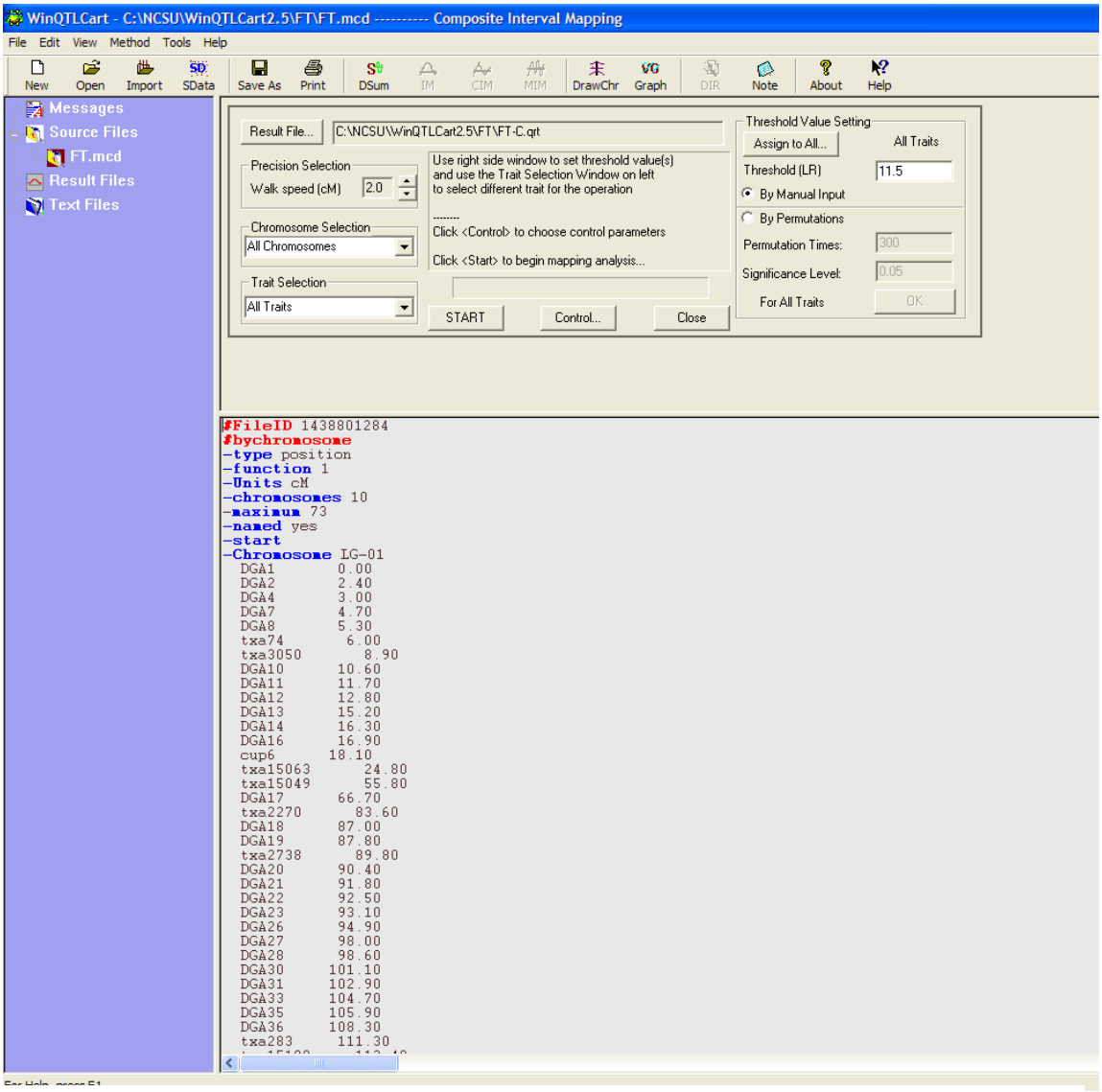


Figure 4.14. Initial QTL mapping parameters.

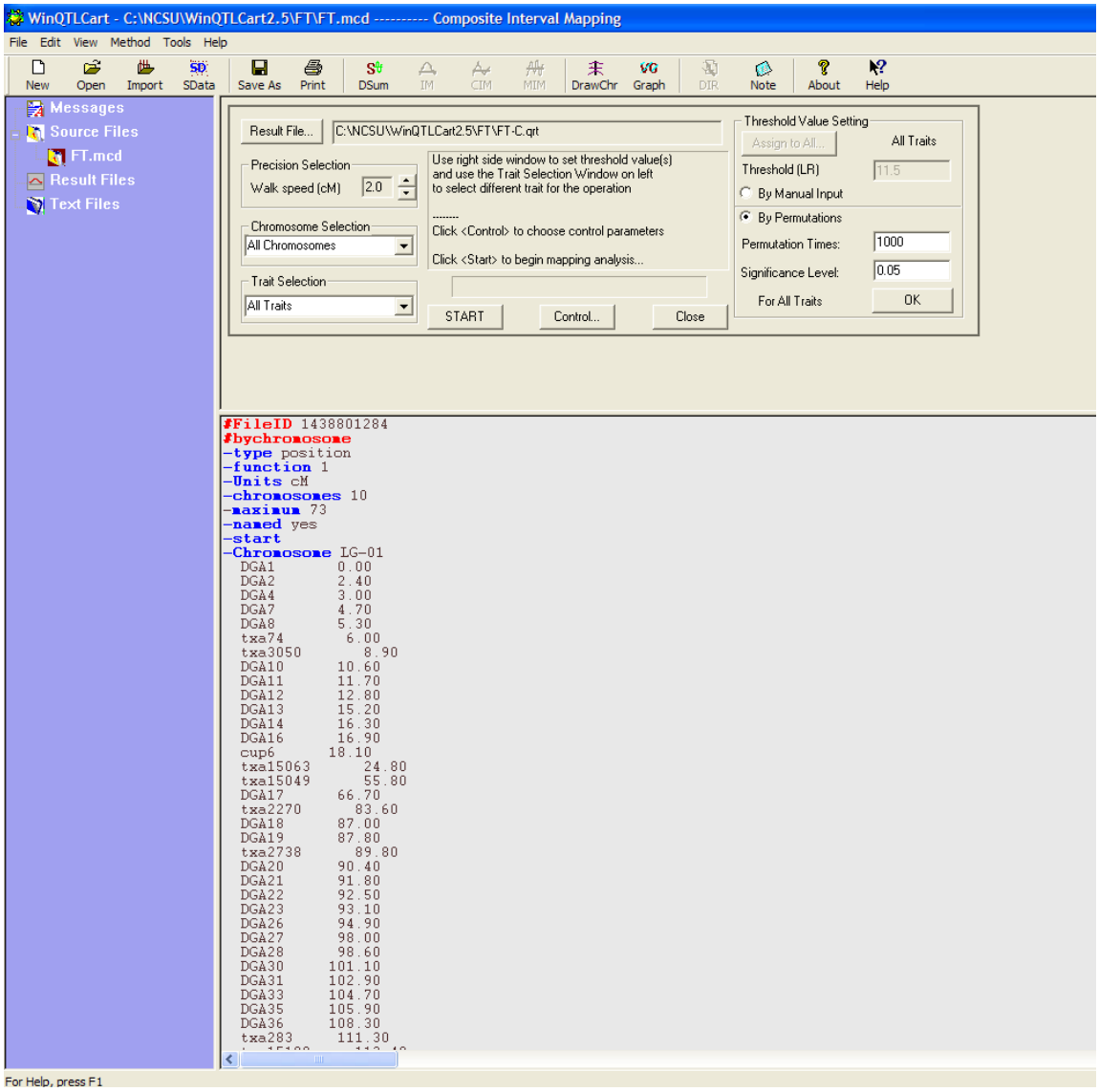


Figure 4.15. QTL significance threshold setup.

Table 4.3. Permutation test for determination of the LR threshold at 95% confidence.

Rank	K1	K2	N1	N2	E1	E2	I1	I2
1	24.06	23.80	26.62	22.07	22.42	23.36	28.53	30.26
2	21.74	22.32	23.97	21.68	20.14	22.60	27.87	27.69
3	21.37	20.27	22.45	21.06	19.61	21.19	27.28	26.75
4	20.46	19.08	22.44	19.12	19.25	20.76	27.17	25.20
5	19.69	18.61	20.50	18.69	18.46	20.74	26.74	24.25
6	17.77	18.29	20.05	18.66	18.26	19.74	25.69	23.97
7	17.72	18.08	19.86	18.48	18.15	18.79	24.76	22.46
8	17.60	17.92	19.46	18.26	17.91	18.25	24.31	20.94
9	17.51	17.80	18.74	17.76	17.85	17.95	24.12	20.75
10	17.50	17.79	18.67	17.23	17.67	17.60	23.91	20.74
11	16.97	17.64	18.33	17.15	17.64	17.47	23.84	20.53
12	16.78	17.33	18.10	17.03	17.48	17.29	23.67	20.33
13	16.19	16.77	17.97	16.84	17.34	17.14	23.50	19.73
14	16.06	16.71	17.94	16.83	17.01	17.00	23.46	19.66
15	16.02	16.59	17.92	16.59	16.87	16.97	22.66	19.32
16	15.99	16.37	17.62	16.41	16.86	16.61	22.66	19.17
17	15.94	16.35	17.60	16.40	16.82	16.54	22.25	18.57
18	15.92	16.23	17.26	16.39	16.70	16.06	22.18	18.47
19	15.72	16.12	17.05	16.07	16.56	16.05	21.44	18.27
20	15.44	16.05	16.91	16.02	16.54	15.99	21.25	17.37
21	15.34	15.79	16.85	16.00	16.43	15.99	21.13	17.23
22	15.32	15.77	16.71	15.97	16.35	15.96	21.12	16.99
23	15.26	15.60	16.66	15.84	16.25	15.71	20.89	16.87
24	15.18	15.47	16.58	15.75	16.23	15.64	20.88	16.80
25	15.11	15.43	16.57	15.69	16.08	15.62	20.83	16.72
26	15.07	15.40	16.38	15.48	16.04	15.48	20.28	16.69
27	15.05	15.39	16.24	15.43	15.86	15.43	19.93	16.57
28	15.01	15.23	16.23	15.42	15.84	15.42	19.19	16.50
29	14.89	15.23	16.11	15.39	15.81	15.34	18.99	16.45
30	14.86	15.19	15.99	15.16	15.72	15.33	18.94	16.18
31	14.82	15.08	15.95	15.15	15.71	15.22	18.70	15.81
32	14.65	15.08	15.71	15.00	15.68	15.22	18.68	15.76
33	14.64	15.07	15.69	14.93	15.24	15.20	18.66	15.74
34	14.48	15.06	15.61	14.82	15.15	15.15	18.30	15.70
35	14.48	14.94	15.57	14.66	15.11	15.12	18.29	15.62
36	14.46	14.88	15.57	14.58	14.96	15.06	18.25	15.53
37	14.38	14.76	15.55	14.56	14.91	14.97	18.21	15.50
38	14.37	14.76	15.19	14.54	14.87	14.94	18.12	15.43

Table 4.3, continued.

39	14.32	14.70	15.01	14.51	14.86	14.79	18.01	15.42
40	14.27	14.70	14.95	14.51	14.86	14.71	17.82	15.36
41	14.24	14.69	14.94	14.45	14.73	14.71	17.64	15.32
42	14.23	14.68	14.84	14.43	14.72	14.69	17.59	15.30
43	14.17	14.56	14.80	14.37	14.69	14.68	17.52	15.28
44	14.14	14.52	14.78	14.35	14.60	14.46	17.44	15.24
45	14.11	14.46	14.75	14.34	14.59	14.42	17.40	15.21
46	14.04	14.41	14.65	14.32	14.58	14.38	17.36	15.19
47	13.91	14.37	14.43	14.30	14.55	14.32	17.28	15.17
48	13.90	14.18	14.43	14.29	14.55	14.22	17.19	15.13
49	13.86	14.16	14.35	14.22	14.53	14.18	17.17	15.13
50	13.83	14.12	14.35	14.18	14.30	14.17	16.93	15.05

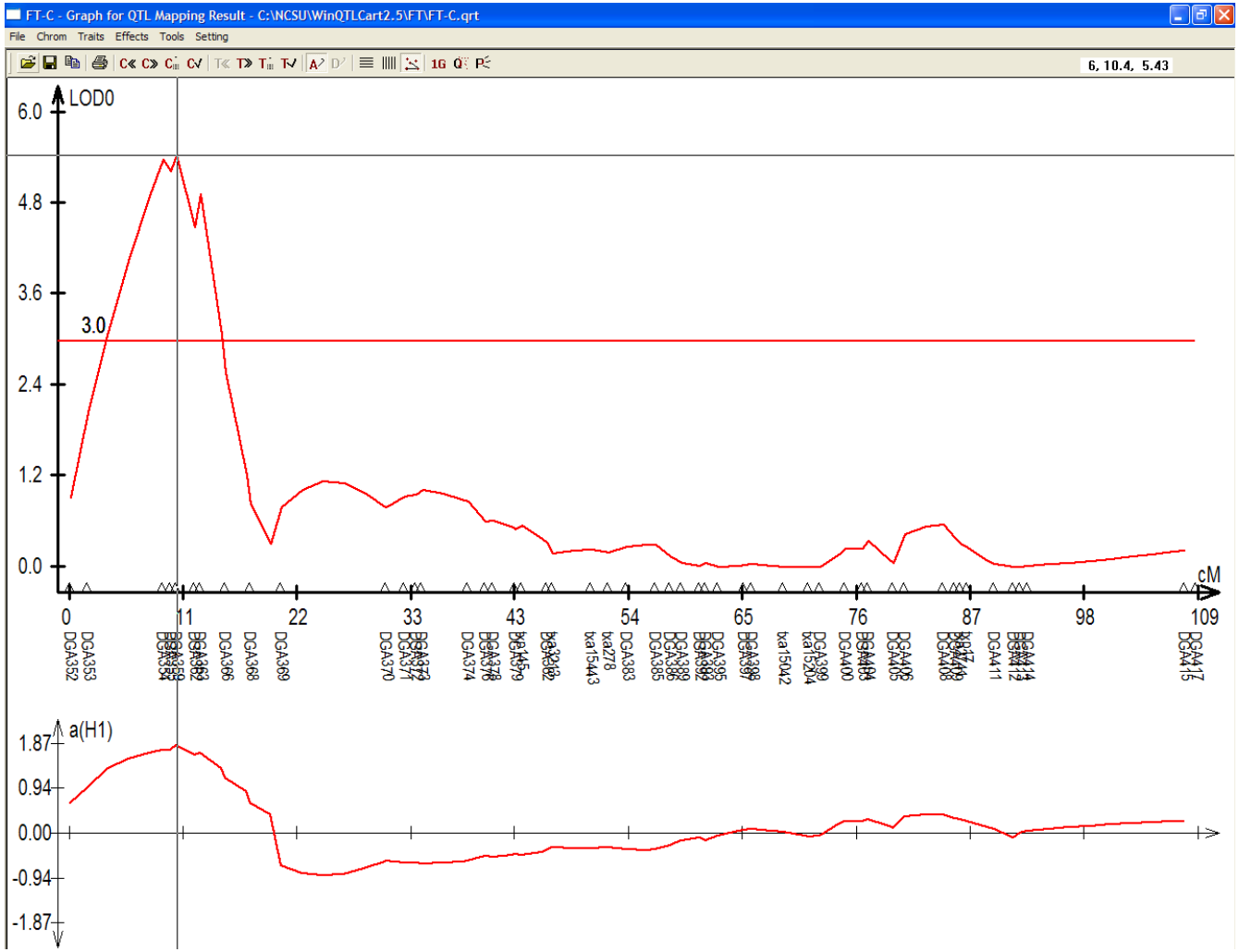


Figure 4.16. Determination of a QTL's LOD score.

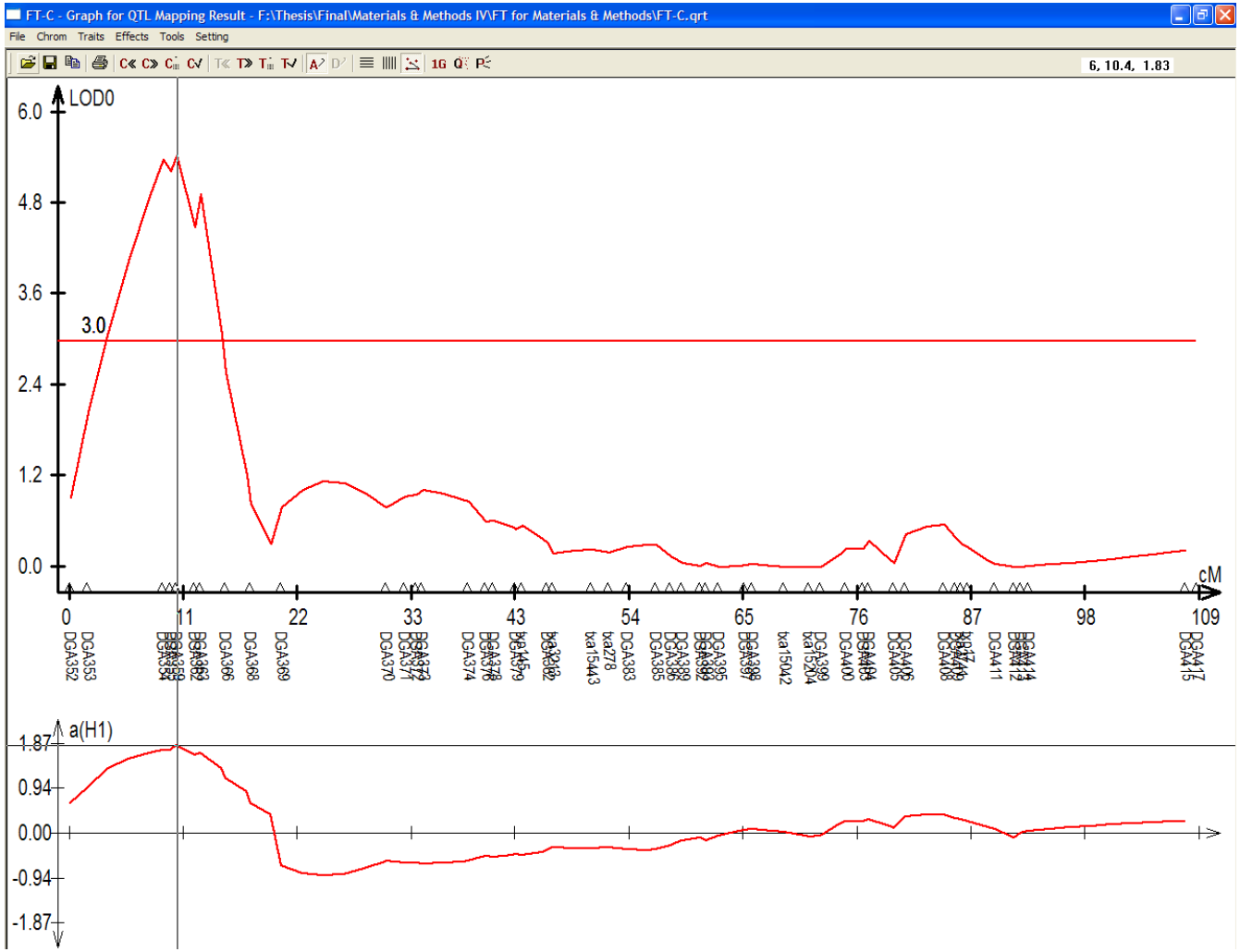


Figure 4.17. Determination of a QTL ‘s additive effect.

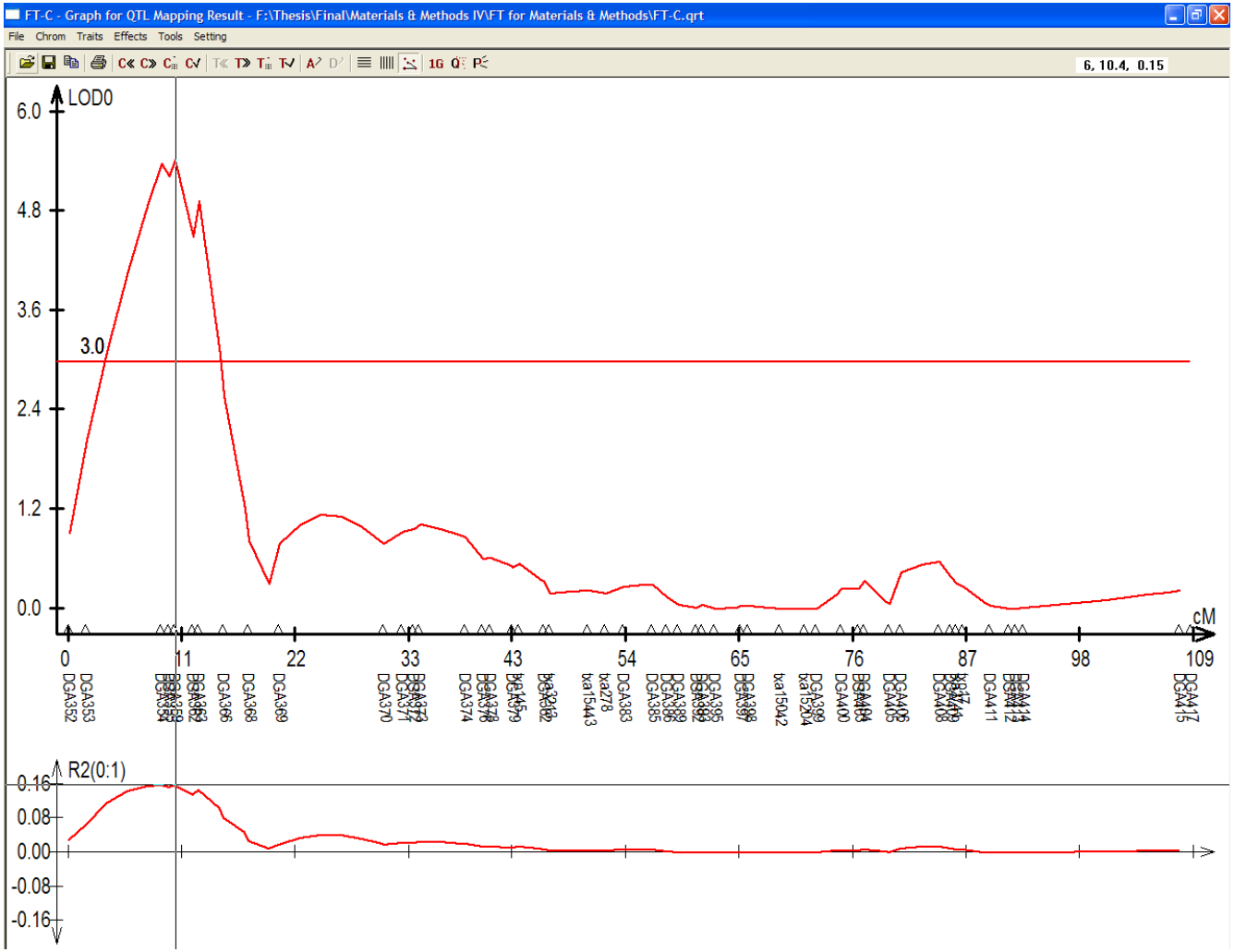


Figure 4.18. Determination of the percent of phenotypic variation explained by a QTL.

The additive effect of a given QTL was determined by the "Show Additive Effect" option in the "Effects" menu of the mapping output window and then positioning the trace hairs on the additive effects curve coincident with the reported LOD for the peak (Fig. 4.17). All additive effects in WinQTL Cartographer were reported in units of the respective trait, and were reported with respect to RTx7000. To determine the percent of the phenotypic variance explained by a given QTL, "Show Values of R2" was selected from the "Effects" menu in the mapping output window and then trace hairs were used to determine the phenotypic variance at the reported LOD for the peak (Fig. 4.18). The value for the phenotypic variance was reported in the upper right corner of the mapping window; in Figure 4.18 the phenotypic variance for the given QTL was 15%.

Multiple Interval Mapping (MIM) QTL Analysis

WinQTL Cartographer V2.5 (Wang et al., 2007) was used to create a source file for a given trait and then "MIM" was selected in the main window. In the resulting window an individual trait was selected; in this example "FTK" and then the "MIM" option was selected (Fig. 4.19). Within the MIM main window a model was created by using the "New Model" function (Fig. 4.20), followed by "MIM forward search method" in the subsequent window (Fig. 4.21). MIM analysis was conducted at 95% confidence and with a default walk speed of 1 cM by selecting the options "Score – 0.05 significant level" and "1" in the "Select Parameters" window (Fig. 4.22). The analysis output was displayed in the MIM main window (Fig. 4.23). In the example analysis, examination of Figure 4.24 shows that MIM detected two QTL for the trait; one on LG-01 with the main

peak centered at 88.0 cM, and the second on LG-08 with the main peak centered at 58.0 cM. Additive effects (in units of the trait measured) were determined by examining the "Additive" line in the main MIM window. In the example, the QTL on LG-01 had an additive effect of -1.1 days and the QTL on LG-08 had an additive effect of -1.6 days (the RTx7000 allele decreased the magnitude of the trait at this locus, or the BTx642 allele increased the magnitude of the trait at this locus).

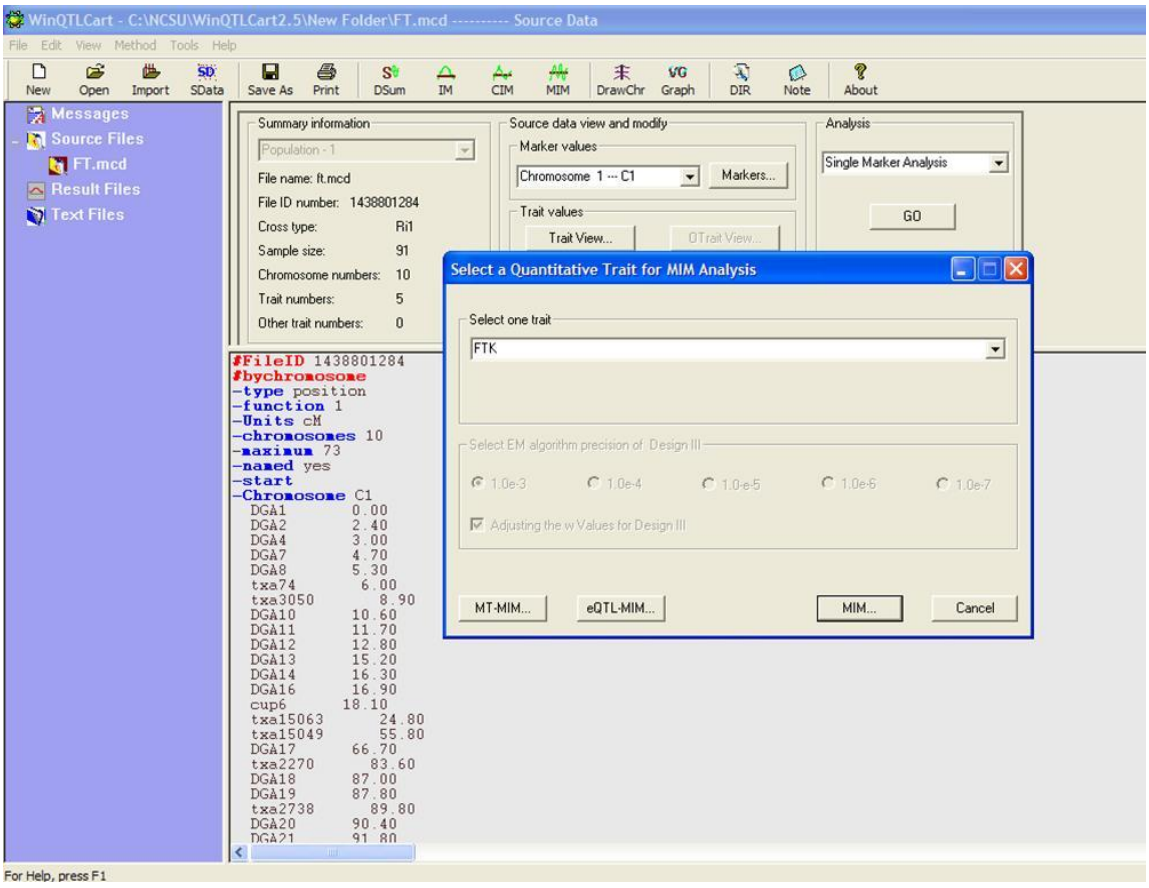


Figure 4.19. Trait selection for MIM analysis.

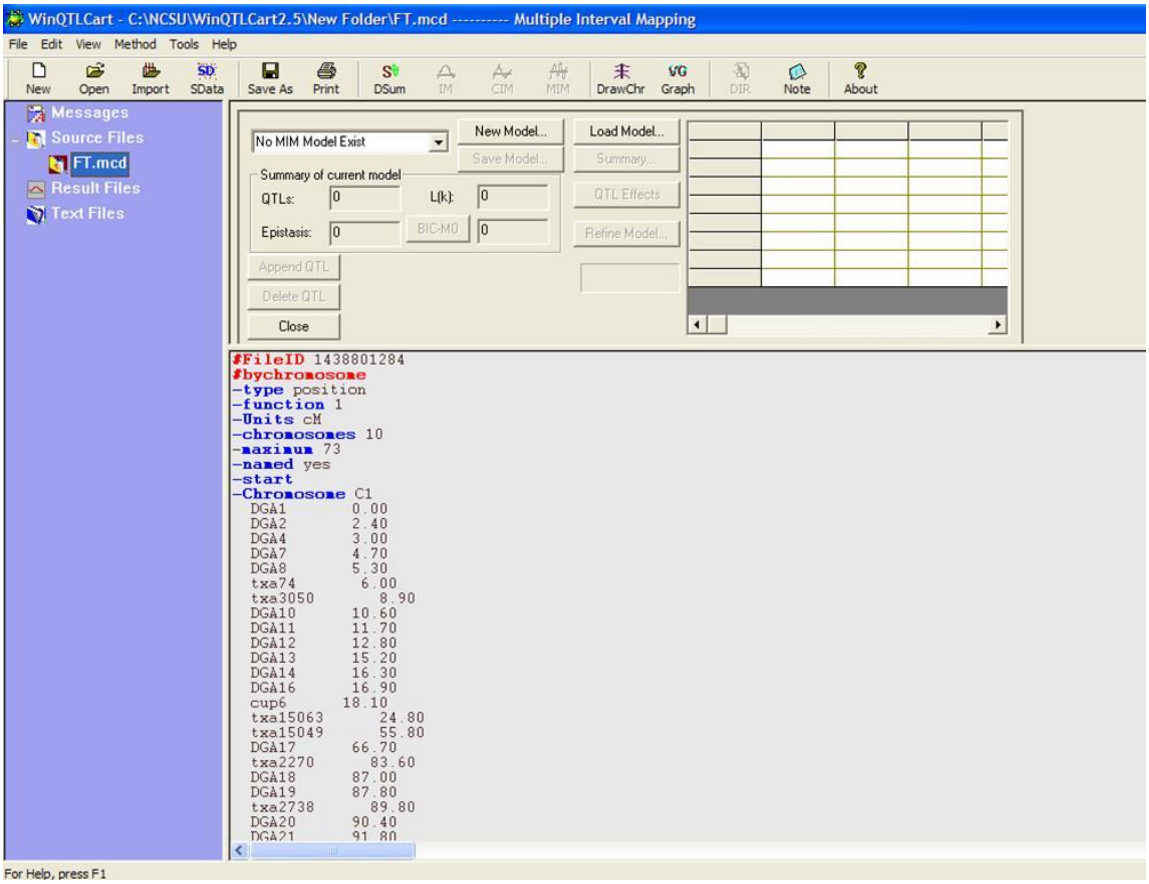


Figure 4.20. MIM analysis main window.

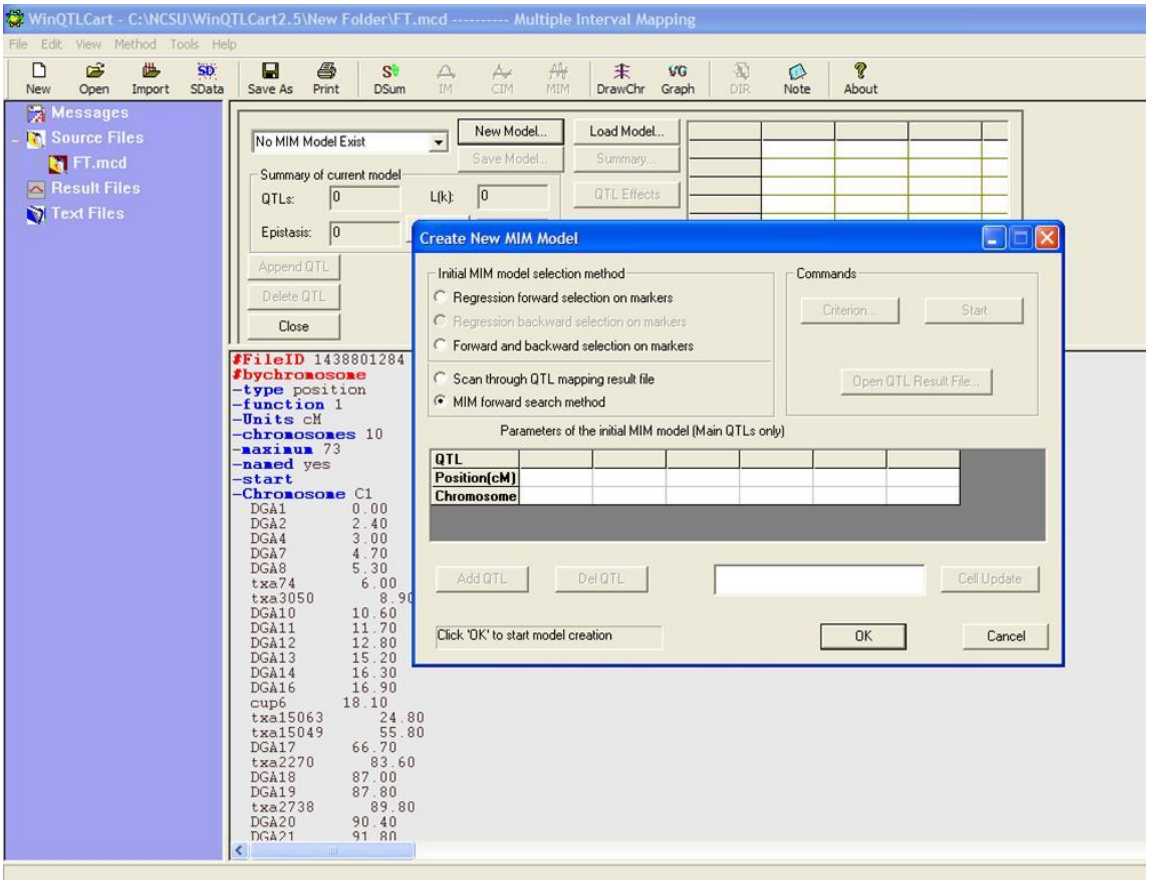


Figure 4.21. MIM model selection.

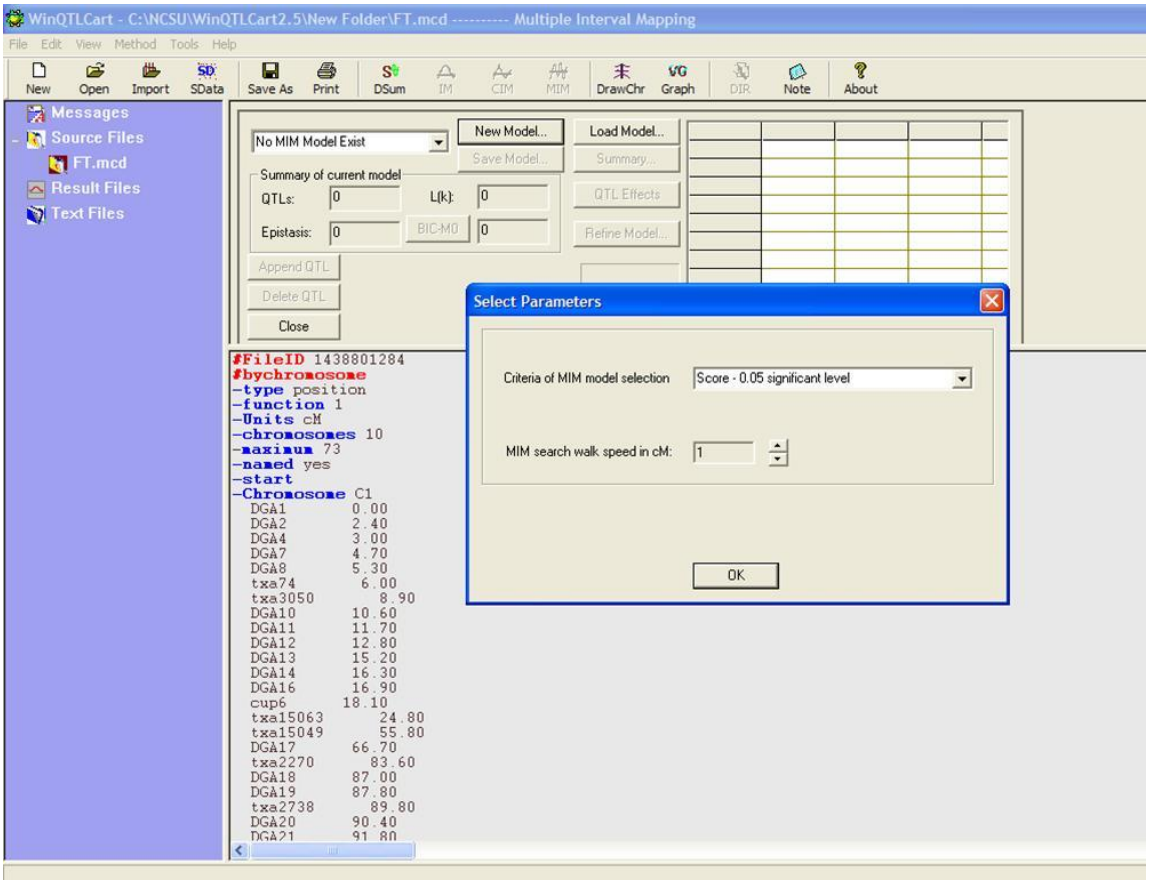


Figure 4.22. MIM parameter selection.

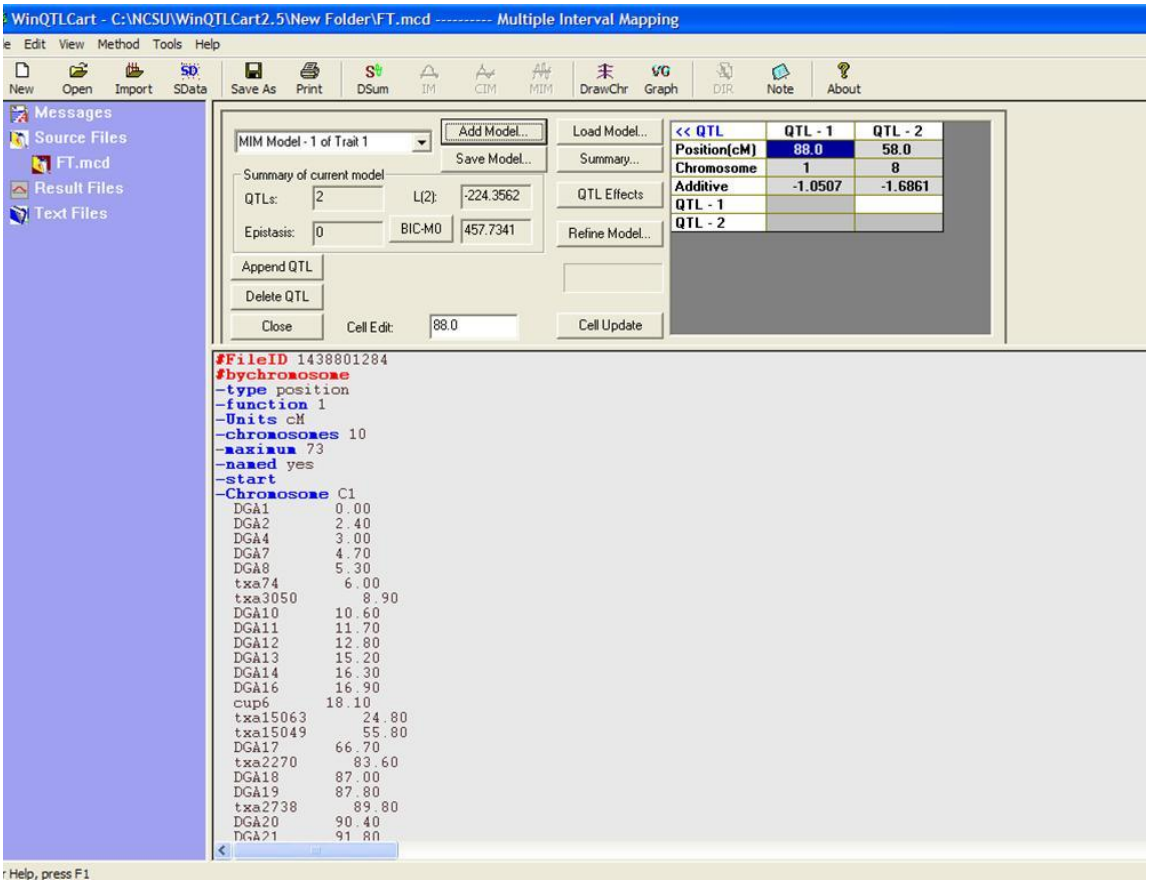


Figure 4.23. MIM analysis result window.

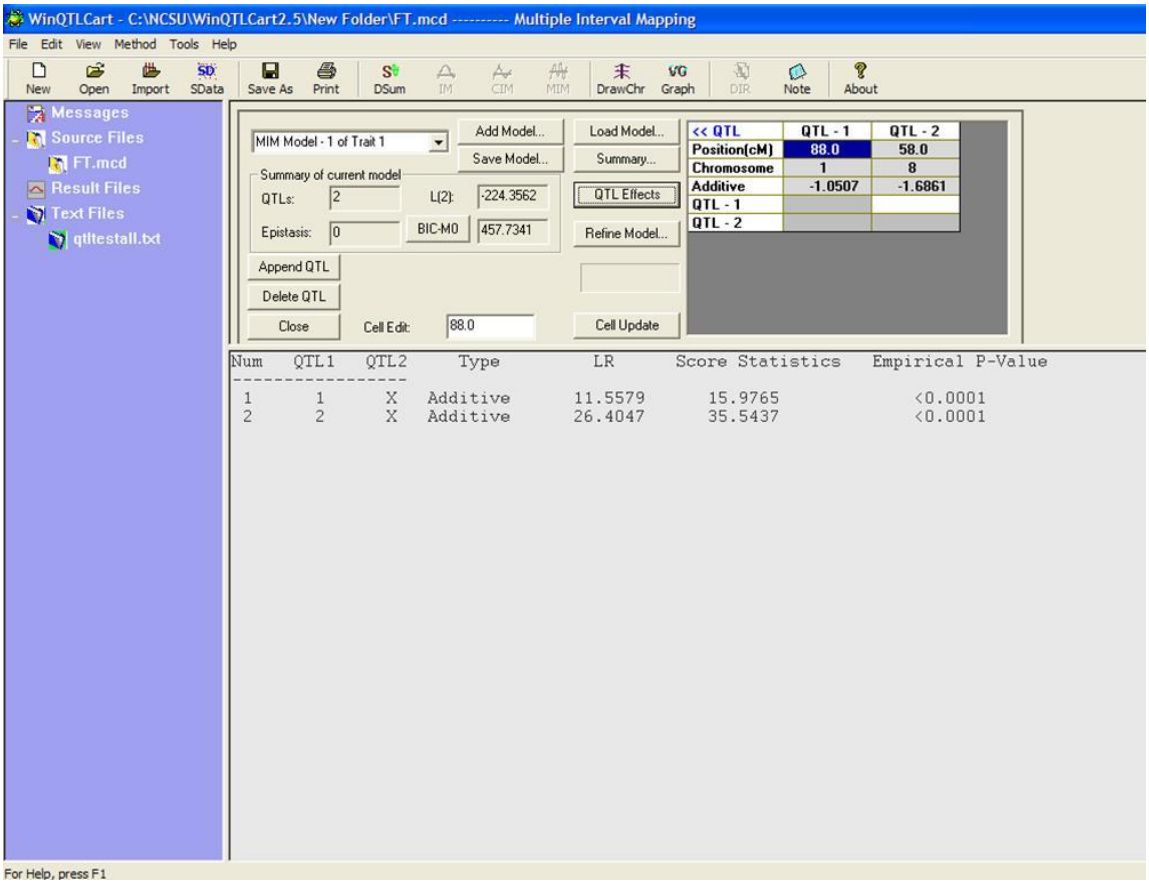


Figure 4.24. MIM epistasis determination.

The likelihood ratio (LR) score for each main QTL was determined in the MIM main window by selecting the "QTL Effects" button. In Figure 4.24 LR scores were 11.56 for the first QTL and 26.40 for the second QTL. LR scores were converted to log of odds scores by multiplying by 0.217. The percent phenotypic variance explained by a given QTL (R^2) was determined by selecting the "Summary" option in the main MIM window. In Figure 4.25 it is shown that the first QTL explained 9.0% of the phenotypic variance and the second explained 23.0%. QTL width was determined by opening the

graph file through the “Summary” option in the main window and then determining peak width in a manner consistent with CIM QTL width reporting, discussed previously.

Epistatic effects between main QTL were examined by selecting the “Refine Model” option in the main window and then in the subsequent window selecting “Searching for New QTL” and “QTL Interactions” using the same criteria as for detecting main QTL. Epistatic interactions were summarized by selecting the “QTL Effects” option in the MIM main window (Fig. 4.24). In the example, both QTL had additive effects, but there was no evidence of epistasis between them at 95% confidence.

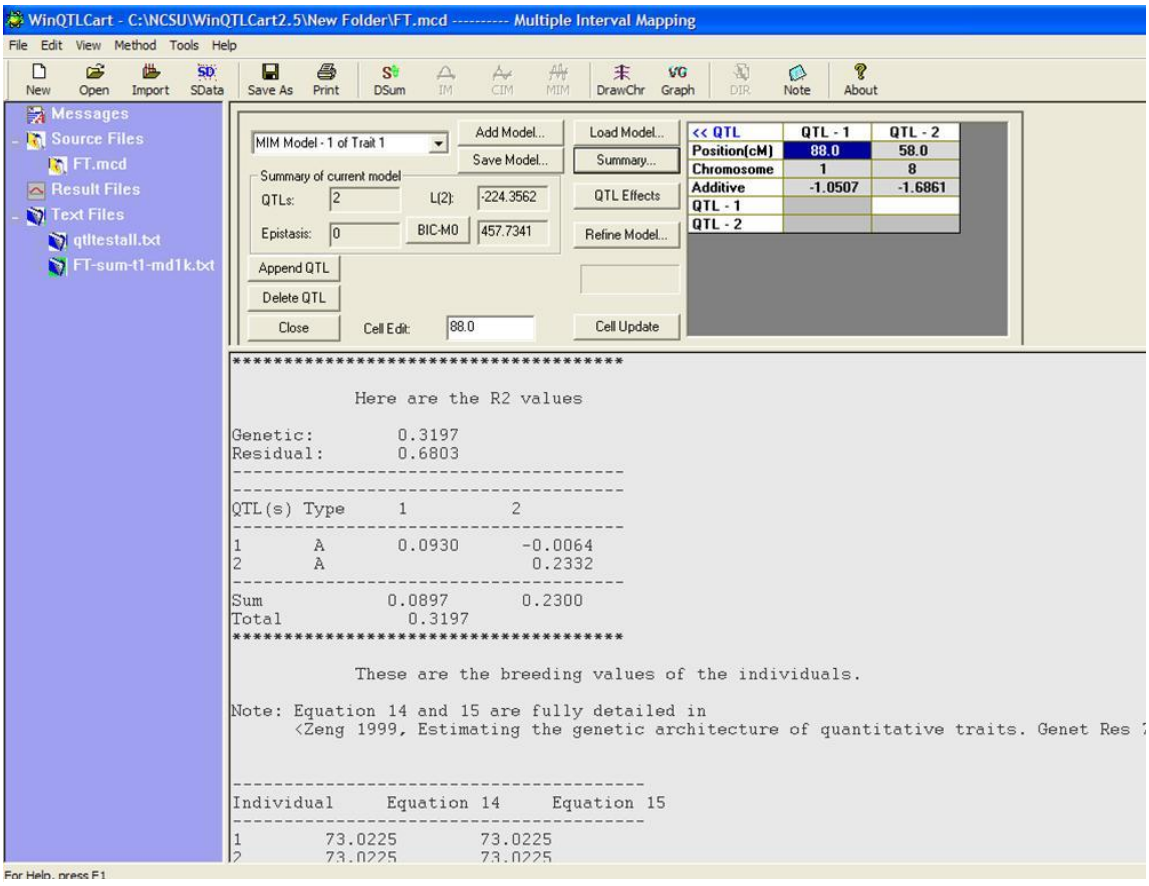


Figure 4.25. MIM determination of phenotypic variance.

CHAPTER V

GENETIC BASIS OF HEIGHT AND FLOWERING TIME VARIATION IN BTx642 AND RTx7000[1]

Introduction

Sorghum genotypes from Africa are often very tall and late flowering when grown in central Texas and further north. During the early 1900's farmers and breeders identified and began using sorghum genotypes found among the introduced accessions that were shorter and that flowered earlier because these materials increased grain yield and facilitated grain harvesting (Quinby, 1974). This task of breeding for genotypes that were dwarf and photoperiod insensitive was ultimately charged to the Texas Agricultural Experiment Station and the USDA Sorghum Conversion Program in 1960 (Stephens et al., 1967). Four maturity loci *Ma1*, *Ma2*, *Ma3*, and *Ma4*, and four height loci *Dw1*, *Dw2*, *Dw3*, and *Dw4*, were identified and characterized by Karper and Quinby (1945) and Karper and Quinby (1954), respectively. Examination of F_1 progeny and segregation analyses of F_2 and F_3 generations derived from genotypes differing in height and/or flowering time were used to deduce the number of genes involved in the respective phenotypes. Later, two additional maturity loci were discovered, *Ma5* and *Ma6* (Rooney and Aydin, 1999).

[1]This chapter was reviewed by Stanley Luck, Ph.D., a statistician and QTL expert from Dupont, Wilmington, DE, and Petra Wolters, Ph.D., a plant geneticist, from Dupont, Wilmington, DE.

In general recessive *Ma* alleles cause earlier flowering, with *Ma1* having the largest effect on flowering time under field conditions in Plainview, Texas (Quinby, 1974). With respect to plant height, the four loci are considered brachytic, meaning they primarily affect internode length. A recessive allele at any of the *Dw* loci causes dwarfing, and in most backgrounds the more recessive *Dw* alleles present, the shorter the plant (Quinby, 1974). Flowering time in sorghum is affected by day length, temperature (Major et al., 1990), and gibberellins (Lee et al., 1998). Sorghum is a short day plant. Photoperiod sensitive genotypes flower earlier in short days compared to long days. Photoperiod control involves the interaction of output from the circadian clock (Bunning, 1960) and diurnal light signals transmitted through photoreceptors such as the phytochromes, cryptochromes, and phototropins (Casal, 2000). Of the six maturity loci, only *Ma3* has been cloned. *Ma3* encodes phytochrome B and is located on chromosome 1 (Childs et al., 1997). A QTL corresponding to *Ma4* was reported to be on the end of chromosome 10 (Peng et al., 1999) and *Ma1* has been mapped to chromosome 6 (Lin et al., 1995; Klein at al., 2008). *Ma1* is linked to *Dw2* (Quinby 1974). Of the four height genes, only *Dw3* has been cloned. This gene is located on chromosome 7 and encodes a phosphoglycoprotein auxin efflux carrier that is homologous to phosphoglycoprotein 1 in Arabidopsis (Multani et al., 2003).

Flowering time, or anthesis, is typically measured in sorghum as the number of days after emergence when half of a given row of plants of a specific genotype has shed pollen from one half of the panicle (Quinby, 1974). Control of flowering time is an important adaptation to drought because the reproductive phase is especially sensitive to

water limitation. In environments subject to late season drought, early flowering plants can complete their growth cycle under favorable well-watered conditions and avoid the negative impact of drought. RTx7000 (Caprock) is a kafir-milo grain sorghum derived from Blackhull kafir, a photoperiod insensitive line that contains a recessive form of *Ma1* (*ma1*-kafir allele) (Quinby, 1974; Klein et al., 2008). RTx7000 is a three dwarf plant with the genotype *dw1, Dw2, dw3, dw4* (Quinby, 1974). BTx642 is a BC_1 line that was derived from crossing IS12555 (Durra) to BTx406, an early flowering four dwarf genotype used in the sorghum conversion program (Klein et al., 2008). BTx406 is early flowering due in part to the presence of a recessive *ma1* allele from Early White Milo. BTx406 is short due to recessive *dw1-dw4* alleles. During conversion, BTx642 acquired *ma1* and probably most or all of the *dw1-dw4* recessive alleles from BTx406.

Plant height is typically measured as the length of the stalk from the base of the plant to the flag leaf (Quinby, 1974). This measurement is useful for comparing overall differences in the average lengths of all internodes in plants with similar flowering time plus the length of the last leaf sheath. The stalk itself is composed of two chief components: 1) the leaf sheaths which emerge from the vegetative shoot apical meristem at each internode and function in both physical support for the leaf and internodes during elongation and as a pathway for water and solute movement between the leaf and the stem, and 2) the stem (or culm) which provides structural support for the overall plant and serves as a vascular conduit between leaves and roots, and can function as an assimilate sink.

The analysis of BTx642 and RTx7000 phenology showed that these genotypes differed in height and flowering time. These traits also segregated in the RIL population derived from BTx642 and RTx7000. Variation in flowering time or height may have an impact on post-flowering drought tolerance, shoot biomass, and yield. Therefore, a QTL study was done to understand the number and location of the genetic loci that modulate these traits in this population.

Results

Stalk Length

Stalk length was measured from the base of the plant to the junction of the leaf blade and sheath of the flag leaf in plants in the flowering phase. Stalk length in the BTx642 x RTx7000 RIL population was examined in well-watered environments at anthesis. Growth environments and times of measurement included plants grown in greenhouse pots and in field conditions in 2009 and 2010.

Parental mean heights were similar when plants were grown in greenhouse pots (Table 5.1). In field conditions, RTx7000 stalk length was greater than BTx642 (75.8 cm vs. 60.3 cm in 2009 and 69.8 cm vs. 52.7 cm in 2010). Additionally, stalk length was roughly 20-25% greater in plants grown under field conditions relative to plants grown in greenhouse pots. The range of RIL stalk lengths exceeded parental stalk length values. Genetic and genetic x environment effects were significant across years in the field and genetic, environment, and genetic x environment effects were significant across locations ($P<0.001$) (Table 5.2). Broad sense heritability of stalk length was high at 0.95 across years and 0.86 across locations. Across years, genetic variation contributed

82.3% to total variation, environment 0.1%, and genetic x environment 9.6%, whereas across locations 38.9%, 37.6%, and 13.7%, respectively, contributed to total variation.

Table 5.1. Stalk length for BTx642 x RTx7000 parental and recombinant inbred lines (RILs) in greenhouse pots and field 2009 and 2010.

Stalk Length (cm)	BTx642	RTx7000	RIL Population		
	Mean	Mean	Mean (SD[†])	Min.-Max.	CV%[‡]
Greenhouse Pots	43.1	45.8	48.3 (11.6)	28.1-74.5	23.9
Field 2009	60.3	75.8	66.2 (14.4)	41.8-104.4	21.8
Field 2010	52.7	69.8	65.5 (17.8)	38.9-102.8	27.1

[†]Standard deviation.

[‡]Coefficient of variation.

Table 5.2. Analysis of variance across well-watered environments for stalk length in the BTx642 x RTx7000 RIL population.

Source of variation	Across years in the field[†]					Across locations[‡]				
	df	MS[§]		SS[¶]	CoV[#]	TV%[†]	df	MS[§]		SS[¶]	CoV[#]	TV%[†]
Rep	1	1.98E-01		2.06E-06	1.19E-01	<0.1	1	3.13E+00		2.79E-05	1.96E-01	<0.1
Entry	89	9.63E+02	***	8.92E-01	2.23E+02	82.3	89	7.48E+02	***	5.92E-01	1.52E+02	38.9
Environment	1	2.59E+01		2.70E-04	2.66E-01	0.1	1	2.63E+04	***	2.34E-01	1.47E+02	37.6
Entry*Environment	89	7.36E+01	***	6.81E-02	2.61E+01	9.6	89	1.45E+02	***	1.14E-01	5.37E+01	13.7
Error	178	2.15E+01		3.99E-02	2.15E+01	7.9	177	3.79E+01		5.97E-02	3.79E+01	9.7
Mean (cm)		65.7						57.0				
Minimum (cm)		38.1						25.5				
Maximum (cm)		111.7						111.7				
Coefficient of variation (%)		24.9						31.1				
LSD (0.05)		1.4						1.8				
Broad sense heritability		0.95						0.86				

[***]Indicates significance at the 0.001 probability level.

[†]2009 and 2010.

[‡]Field 2010 and greenhouse pots .

[§]Mean square.

[¶]Proportion of total sums of squares.

[#]Covariate value.

[††]Percentage of total variation.

Stalk length QTL derived by composite interval mapping (CIM) were detected in all environments and at both growth stages analyzed (Fig. 5.1; Table 5.3). Only QTL with a LOD of 3.10 or greater determined by 1000 permutations at 95% confidence are described. At anthesis stalk length is determined to a much larger degree by internode length. QTL for stalk length at anthesis were mapped to LG-06 and LG-08. On LG-06, RTx7000 alleles increased stalk length and on LG-08 BTx642 alleles increased stalk length.

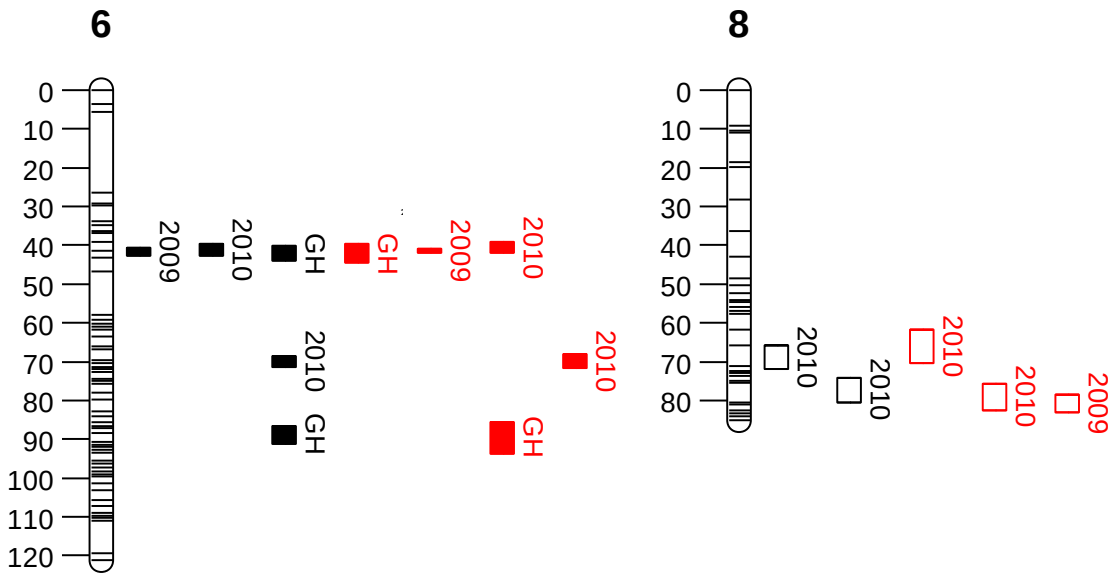


Figure 5.1. Quantitative trait loci (QTL) positions for stalk length in plants grown in greenhouse conditions (GH) and in the field in 2009 and 2010. Filled bars denote QTL where RTx7000 increased the magnitude of the trait; unfilled bars denote QTL where BTx642 increased the magnitude of the trait. CIM-derived QTL are represented as black bars and MIM-derived QTL are represented as red bars. All QTL are shown as the width at one LOD below the QTL peak. Exact QTL locations, LOD scores and thresholds, and phenotypic variances are shown in Tables 5.3 and 5.5.

Table 5.3. Stalk length CIM QTL for BTx642 x RTx7000 RILs in greenhouse pots and field 2009 and 2010.

Env.	LG[†]	Peak[‡]	LOD	LOD-1[§]	LOD-2[§]	a(H)[¶]	$R^{2\#}$	95% LOD
2009	6	41.5	25.06	40.6 - 42.7	40.1 - 42.8	11.6	0.60	3.10
2010	6	40.9	19.49	39.6 - 42.7	39.1 - 43.0	13.1	0.43	3.22
GH	6	42.3	19.92	40.1 - 44.0	39.7 - 45.0	8.5	0.49	3.27
2010	6	70.5	4.47	68.7 - 71.3	66.9 - 72.8	4.2	0.06	3.22
GH	6	89.1	4.75	86.5 - 91.1	84.2 - 98.4	3.3	0.08	3.27
2010	8	66.0	3.54	65.8 - 71.8	65.7 - 85.0	-3.9	0.04	3.22
2010	8	77.5	3.63	74.1 - 80.5	65.7 - 85.0	-4.2	0.05	3.22

[†]Linkage group.

[‡]QTL peak in cM.

[§]QTL width in cM at either 1 (LOD-1) or 2 (LOD-2) LOD units below the peak.

[¶]Additive effect (cm). Sign is with respect to RTx7000.

[#]Proportion of total phenotypic variance explained by the QTL.

The stalk length QTL on LG-06 from ~40-43 cM was mapped with very high confidence (LOD scores up to 25.1) and this QTL accounted for up to 60% of the variance in stalk length at anthesis. This QTL interval overlaps with a region of the sorghum genome previously identified as encoding *Dw2* (Klein et al., 2008). To further explore this relationship, the linkage of specific markers and variation in stalk length in RILs at anthesis was analyzed (Fig. 5.2). DGA markers mapping from 40.2 Mbp to 42.8 Mbp (~37-43 cM) showed the highest linkage to alleles that cause variation in stalk length. The genotypes and phenotypes of parental lines and RILs with recombinant chromosomes containing breakpoints in this interval were further analyzed and the data summarized in Table 5.4. This analysis showed that the QTL for stalk length on LG-06 maps between DGA markers 377 and 378 (39.0-41.5 cM), a region spanning ~872 Kbp.

This interval corresponds to the region of LG-06 previously shown to encode *Dw2* (Klein et al., 2008).

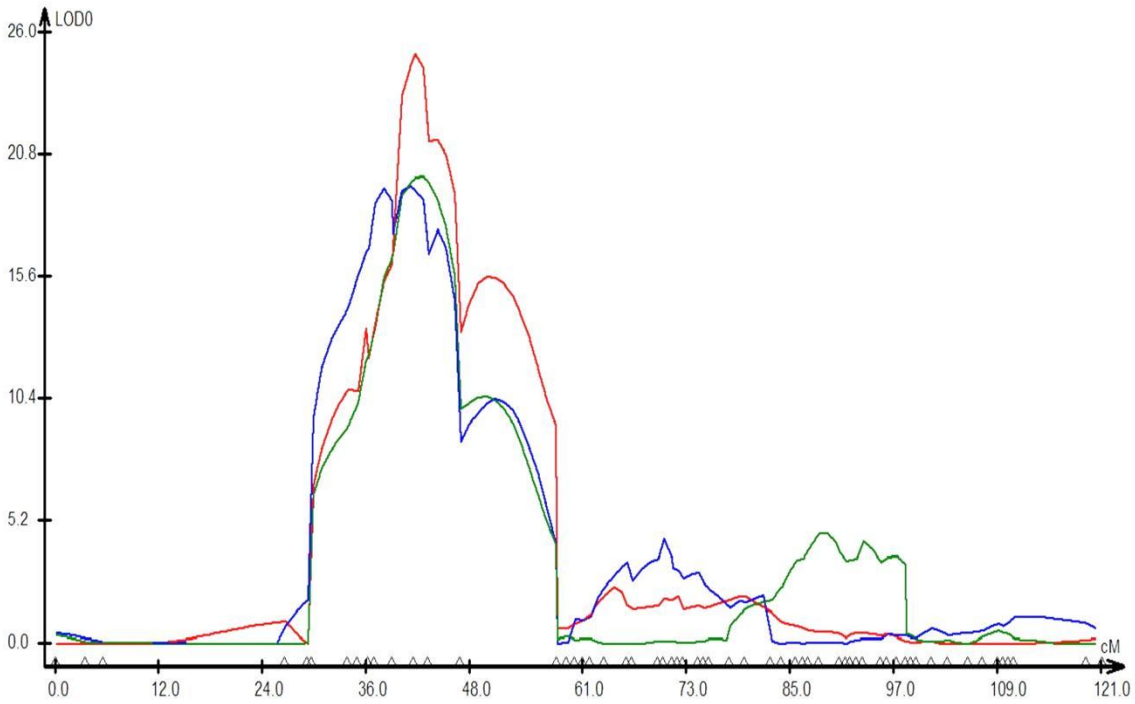


Figure 5.2. Quantitative trait loci (QTL) positions on LG-06 for stalk length in plants grown in greenhouse pots (green line) and in the field in 2009 (red line) and 2010 (blue line) to anthesis. LOD score is plotted as a function of marker position. Recombination break points are shown in Table 5.4.

Table 5.4. LG-06 stalk length QTL haplotype analysis for plants grown in greenhouse conditions and in the field in 2009 and 2010.

Experiment				Marker Physical & Genetic Positions‡		
2009 Mean (SD†)	2010 Mean (SD†)	GH Mean (SD†)	No. of RILs	DG377 41,856,169 39.0	DG378 42,728,446 41.5	DG379 42,795,490 43.1
54.7 (6.7)	51.3 (9.4)	39.5 (7.0)	42	BTx642	BTx642	BTx642
49.3 (-)	40.8 (-)	48.7 (-)	1	BTx642	BTx642	RTx7000
72.5 (-)	68.85 (-)	43.6 (-)	1	BTx642	RTx7000	RTx7000
77.1 (9.9)	80.0 (12.2)	58.2 (8.0)	40	RTx7000	RTx7000	RTx7000
78.7 (-)	80.0 (-)	53.3 (-)	1	RTx7000	RTx7000	BTx642
57.8 (2.8)	68.0 (14.7)	39.7 (3.5)	2	RTx7000	BTx642	BTx642

†Standard deviation.

‡Marker physical position is in base pairs and genetic position is in centimorgans.

Stalk length QTL derived by multiple interval mapping (MIM) were detected in all environments and at both growth stages analyzed (Fig. 5.1; Table 5.5). At 95% confidence, QTL were detected overlapping the most significant QTL detected by composite interval mapping. In Figure 5.1 the position of the main peak is shown, although other, smaller peaks were also detected in other regions (data not shown). No epistatic interactions were detected between QTL at the 95% significance level.

Table 5.5. Stalk length MIM QTL for BTx642 x RTx7000 RILs in greenhouse pots and field 2009 and 2010.

Env.	LG[†]	Peak[‡]	LOD	LOD-1[§]	LOD-2[§]	a(H)[¶]	$R^{2\#}$	95% LOD
GH	6	43.1	16.78	39.7 - 44.5	38.5 - 45.2	7.8	0.52	3.27
2010	6	40.2	18.55	39.1 - 42.0	36.6 - 42.3	12.7	0.57	3.22
2010	6	70.5	3.81	68.1 - 71.7	67.0 - 72.6	4.1	0.13	3.22
GH	6	88.4	3.84	85.7 - 93.7	83.2 - 99.3	3.0	0.12	3.27
2009	6	41.5	23.90	40.8 - 41.9	40.1 - 42.4	11.5	0.65	3.10
2010	8	66.9	5.67	61.7 - 70.3	57.1 - 72.0	-4.2	0.06	3.22
2009	8	81.1	3.23	78.6 - 83.0	71.2 - 85.0	-2.9	0.08	3.10
2010	8	81.0	4.84	75.6 - 82.6	73.7 - 85.0	-4.2	0.06	3.22

[†]Linkage group.

[‡]QTL peak in cM.

[§]QTL width in cM at either 1 (LOD-1) or 2 (LOD-2) LOD units below the peak.

[¶]Additive effect (cm). Sign is with respect to RTx7000.

[#]Proportion of total phenotypic variance explained by the QTL.

Flowering Time

Flowering time of BTx642, RTx7000 and the BTx642 x RTx7000 RIL population was analyzed using plants grown in greenhouse pots in short days (10 hours), and plants grown under field conditions in 2008, 2009, and 2010 as day lengths increased from ~13 hours in April to 14 hours in July. In all environments BTx642 flowered later than RTx7000. BTx642 flowering time ranged from 69.5 DAE under greenhouse conditions to 77.0 DAE under field conditions in 2008 (Table 5.6) Flowering time for RTx7000 was more variable, ranging from 49.8 DAE under greenhouse conditions to 73.0 DAE under field conditions in 2008. RIL means were within parental means in all environments although the flowering times of individual

RILs in the population were both earlier and later than either parent. The range of flowering times among RILs was greatest under greenhouse conditions.

An analysis of variance was conducted to assess the covariates and to calculate broad sense heritability across years in the field and across field and greenhouse conditions. In all cases, genetic, environment, and genetic x environment effects were significant ($P<0.001$) (Table 5.7). Across years in the field, genotype contributed 17.2% to total variation, genotype x environment 4.7%, and environment 72.9%. Across locations, genotype, genotype x environment, and environment contributed 21.3%, 26.5%, and 45.1%, respectively, to total variation. Broad sense heritability was 0.88 across years in the field and 0.68 across locations.

Table 5.6. Flowering time for BTx642 x RTx7000 parental and recombinant inbred lines (RILs) in greenhouse pots and field 2008, 2009, and 2010.

Flowering Time (Days)	BTx642	RTx7000	RIL Population		
	Mean	Mean	Mean (SD[†])	Min.-Max.	CV%[‡]
Greenhouse Pots	69.5	49.8	58.6 (6.6)	46.0-78.9	11.3
Field 2008	77.0	73.0	79.5 (3.5)	73.0-88.0	4.4
Field 2009	71.5	67.5	67.6 (4.3)	59.5-78.5	6.4
Field 2010	70.5	68.5	66.3 (4.7)	58.0-77.0	7.2

[†]Standard deviation.

[‡]Coefficient of variation.

Table 5.7. Analysis of variance across well-watered environments for flowering time in the BTx642 x RTx7000 RIL population.

Source of variation	Across years in the field[†]					Across locations[‡]				
	df	MS[§]		SS[¶]	CoV[#]	TV%[†]	df	MS[§]		SS[¶]	CoV[#]	TV%[†]
Rep	1	2.41E+00		8.18E-05	5.12E-03	<0.1	1	1.29E+01		7.23E-04	4.68E-02	<0.1
Entry	89	8.52E+01	***	2.57E-01	1.25E+01	17.2	89	9.38E+01	***	4.66E-01	1.38E+01	21.3
Environment	2	9.48E+03	***	6.44E-01	5.28E+01	72.9	1	5.28E+03	***	2.95E-01	2.93E+01	45.1
Entry*Environment	178	1.06E+01	***	6.41E-02	3.42E+00	4.7	89	3.88E+01	***	1.93E-01	1.72E+01	26.5
Error	268	3.79E+00		3.44E-02	3.79E+00	5.2	178	4.58E+00		4.55E-02	4.58E+00	7.1
Mean (days)		71.1						62.5				
Minimum (days)		57.1						46.0				
Maximum (days)		88.0						83.3				
Coefficient of variation (%)		10.4						11.3				
LSD (0.05)		0.6						0.6				
Broad sense heritability		0.88						0.68				

[***]Indicates significance at the 0.001 probability level.

[†]2008, 2009, and 2010.

[‡]Field 2010 and greenhouse pots .

[§]Mean square.

[¶]Proportion of total sums of squares.

[#]Covariate value.

[††]Percentage of total variation.

Flowering time QTL were detected in all environments and were localized to LG-01, LG-03, LG-06, LG-08, and LG-10 (Fig. 5.3, Table 5.8). One or more flowering time QTL were detected on LG-01 in the interval from 68.3-94.1 cM in all three years in the field. Flowering time QTL were also detected on LG-03 (29.6-35.2 cM) and on LG-06 in the interval from 26.2 cM to 38.1 cM exclusively under field conditions. Greenhouse flowering time QTL were found co-localized with field QTL on LG-08 and LG-10. One QTL on LG-01 (13.5-20.4 cM) was found exclusively in plants grown under 10 hour days in the greenhouse. BTx642 alleles were responsible for late flowering associated with QTL on LG-01, LG-08, and LG-10, and one QTL on LG-06, whereas RTx7000 alleles caused delayed flowering time due to the remaining QTL on LG-06, as well as one QTL on LG-01 and LG-03.

Flowering time QTL derived by multiple interval mapping (MIM) were detected in all environments (Fig. 5.3; Table 5.9). At 95% confidence, QTL were detected overlapping the most significant QTL detected by composite interval mapping. In Figure 5.3 the position of the main peak is shown, although in some cases other, smaller peaks were also detected in the regions (data not shown). No epistatic interactions were detected between QTL at the 95% confidence level.

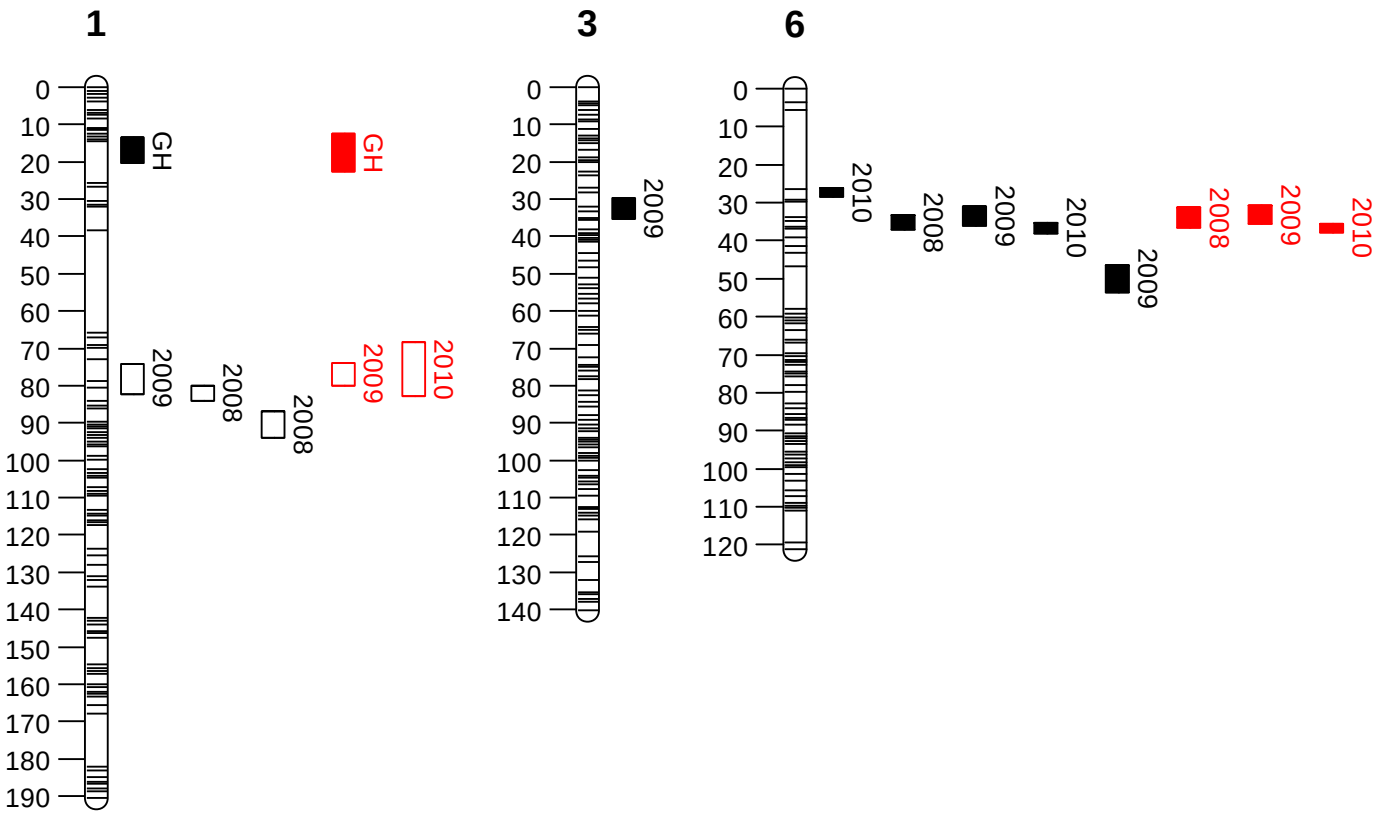


Figure 5.3. Quantitative trait loci (QTL) positions for flowering time in plants grown in greenhouse conditions (GH) and in the field in 2009 and 2010. Filled bars denote QTL where RTx7000 increased the magnitude of the trait; unfilled bars denote QTL where BTx642 increased the magnitude of the trait. CIM-derived QTL are represented as black bars and MIM-derived QTL are represented as red bars. All QTL are shown as the width at one LOD below the QTL peak. Exact QTL locations, LOD scores and thresholds, and phenotypic variances are shown in Tables 5.8 and 5.9.

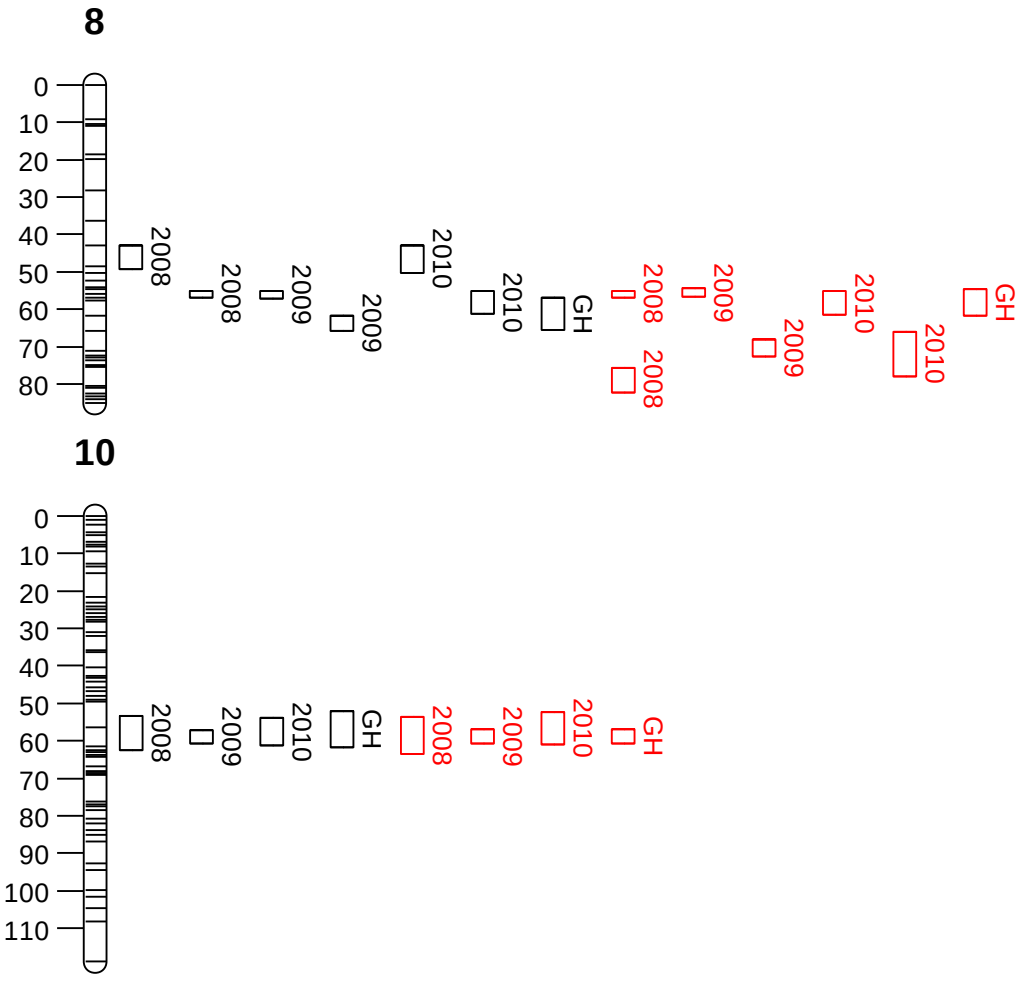


Figure 5.3, continued.

Table 5.8. Flowering time CIM QTL for BTx642 x RTx7000 RILs in greenhouse pots and field 2008, 2009, and 2010.

Env.	LG[†]	Peak[‡]	LOD	LOD-1[§]	LOD-2[§]	a(H)[¶]	$R^{2\#}$	95% LOD
GH	1	14.3	6.44	13.5 - 20.4	12.6 - 22.8	2.2	0.11	3.14
2009	1	80.5	3.92	80.1 - 84.2	79.9 - 85.5	-1.2	0.10	3.07
2008	1	90.9	4.60	86.9 - 94.1	85.4 - 95.4	-1.4	0.12	3.07
2008	1	78.7	4.85	74.1 - 82.2	70.5 - 85.5	-1.5	0.11	3.17
2009	3	33.3	4.92	29.6 - 35.2	24.2 - 37.4	1.5	0.11	3.17
2010	6	26.5	8.19	26.2 - 28.5	26.1 - 29.1	2.5	0.22	3.05
2008	6	34.9	6.31	33.2 - 37.2	31.3 - 38.2	1.8	0.16	3.07
2009	6	34.8	6.31	30.9 - 36.0	26.8 - 36.5	2.0	0.15	3.17
2010	6	36.9	12.17	35.3 - 38.1	34.6 - 38.4	3.1	0.30	3.05
2009	6	47.0	4.10	46.6 - 53.5	46.4 - 55.8	-1.7	0.09	3.17
2008	8	46.1	3.25	42.9 - 49.3	42.7 - 50.2	-1.3	0.11	3.07
2010	8	46.5	4.92	42.9 - 50.3	42.9 - 50.3	-1.8	0.13	3.05
2008	8	55.8	5.90	55.1 - 56.9	54.4 - 58.8	-1.5	0.15	3.07
2009	8	55.9	6.59	55 - 57.1	52.5 - 57.8	-1.8	0.16	3.17
2010	8	56.9	7.09	55.1 - 61.2	51.8 - 64.1	-2.0	0.16	3.05
GH	8	59.8	5.98	57 - 65.6	54.3 - 65.8	-2.1	0.10	3.14
2009	8	64.4	5.29	61.6 - 65.8	61.6 - 65.8	-1.7	0.14	3.17
2008	10	59.4	4.70	53.4 - 62.6	50.5 - 65.7	-1.8	0.13	3.07
2009	10	59.4	8.53	57.2 - 60.8	56.1 - 61.5	-3.1	0.23	3.17
2010	10	58.8	6.14	53.9 - 61.3	51.8 - 61.9	-2.6	0.15	3.05
GH	10	58.3	9.20	52.1 - 61.6	51.1 - 62.4	-3.5	0.19	3.14

[†]Linkage group.

[‡]QTL peak in cM.

[§]QTL width in cM at either 1 (LOD-1) or 2 (LOD-2) LOD units below the peak.

[¶]Additive effect (days). Sign is with respect to RTx7000.

[#]Proportion of total phenotypic variance explained by the QTL.

Table 5.9. Flowering time MIM QTL for BTx642 x RTx7000 RILs in greenhouse pots and field 2008, 2009, and 2010.

Env.	LG[†]	Peak[‡]	LOD	LOD-1[§]	LOD-2[§]	a(H)[¶]	$R^{2\#}$	95% LOD
GH	1	16.0	4.51	12.5 - 22.7	10.3 - 26.9	2.7	0.20	3.14
2009	1	76.7	7.44	73.8 - 80.1	73.2 - 82.6	-1.5	0.10	3.17
2010	1	70.8	4.02	68.3 - 82.9	67.1 - 84.8	-1.6	0.08	3.05
2008	6	34.9	4.33	31.3 - 36.6	29.2 - 38.3	1.6	0.08	3.07
2009	6	34.9	9.79	30.7 - 35.6	30.0 - 36.3	2.5	0.07	3.17
2010	6	37.0	12.81	35.6 - 37.8	34.7 - 38.1	3.4	0.24	3.05
2008	8	55.8	5.14	55.2 - 56.8	54.4 - 59.3	-1.5	0.10	3.07
2009	8	55.8	7.74	54.3 - 56.7	51.2 - 57.5	-2.0	0.10	3.17
2010	8	55.9	5.97	55.2 - 61.4	53.6 - 64.4	-1.9	0.09	3.05
GH	8	55.9	8.08	54.6 - 61.6	53.7 - 65.4	-2.9	0.14	3.14
2009	8	71.2	5.90	68.1 - 72.7	66.4 - 73.7	-1.7	0.10	3.17
2010	8	71.2	3.51	66.0 - 77.9	66.0 - 81.4	-1.5	0.09	3.05
2008	8	78.6	4.43	75.6 - 82.4	71.3 - 85.0	-1.5	0.10	3.07
2008	10	58.4	3.38	53.6 - 63.5	50.6 - 66.7	-1.7	0.07	3.07
2009	10	59.3	9.25	56.9 - 60.8	54.6 - 61.2	-3.2	0.19	3.17
2010	10	58.3	5.73	52.3 - 61.0	50.6 - 64.8	-2.7	0.10	3.05
GH	10	58.9	14.65	56.9 - 60.6	56.4 - 61.1	-4.0	0.39	3.14

[†]Linkage group.

[‡]QTL peak in cM.

[§]QTL width in cM at either 1 (LOD-1) or 2 (LOD-2) LOD units below the peak.

[¶]Additive effect (days). Sign is with respect to RTx7000.

[#]Proportion of total phenotypic variance explained by the QTL.

The flowering time QTL on LG-10 explained up to 39% of the variation in flowering time observed under short day greenhouse conditions (Table 5.9). A sorghum homolog of *CONSTANS* (*CO*) or *Hd1* in rice, a gene involved in flowering time regulation is located on SBI-10 within the region spanned by this QTL. Therefore the flowering time QTL on LG-10 was further delimited through analysis of RILs that contained recombinant chromosomes with breakpoints within the interval spanned by the QTL. Figure 5.4 shows that markers DG541 – DG543 exhibit the highest linkage to the QTL's peak. Further analysis of the region identified RILs with four different haplotypes spanning the region from DG541 to DGA543; 62 RILs had RTx7000 DNA spanning the interval, 20 RILs had BTx642 spanning the interval, and the remaining eight RILs contained different types of recombinant chromosomes. This region spans a physical distance of approximately 4.5 Mb and a genetic distance of 6.2 cM and encodes the sorghum ortholog of CO (Hd1) at 12,275,128 Mbp on SBI-10.

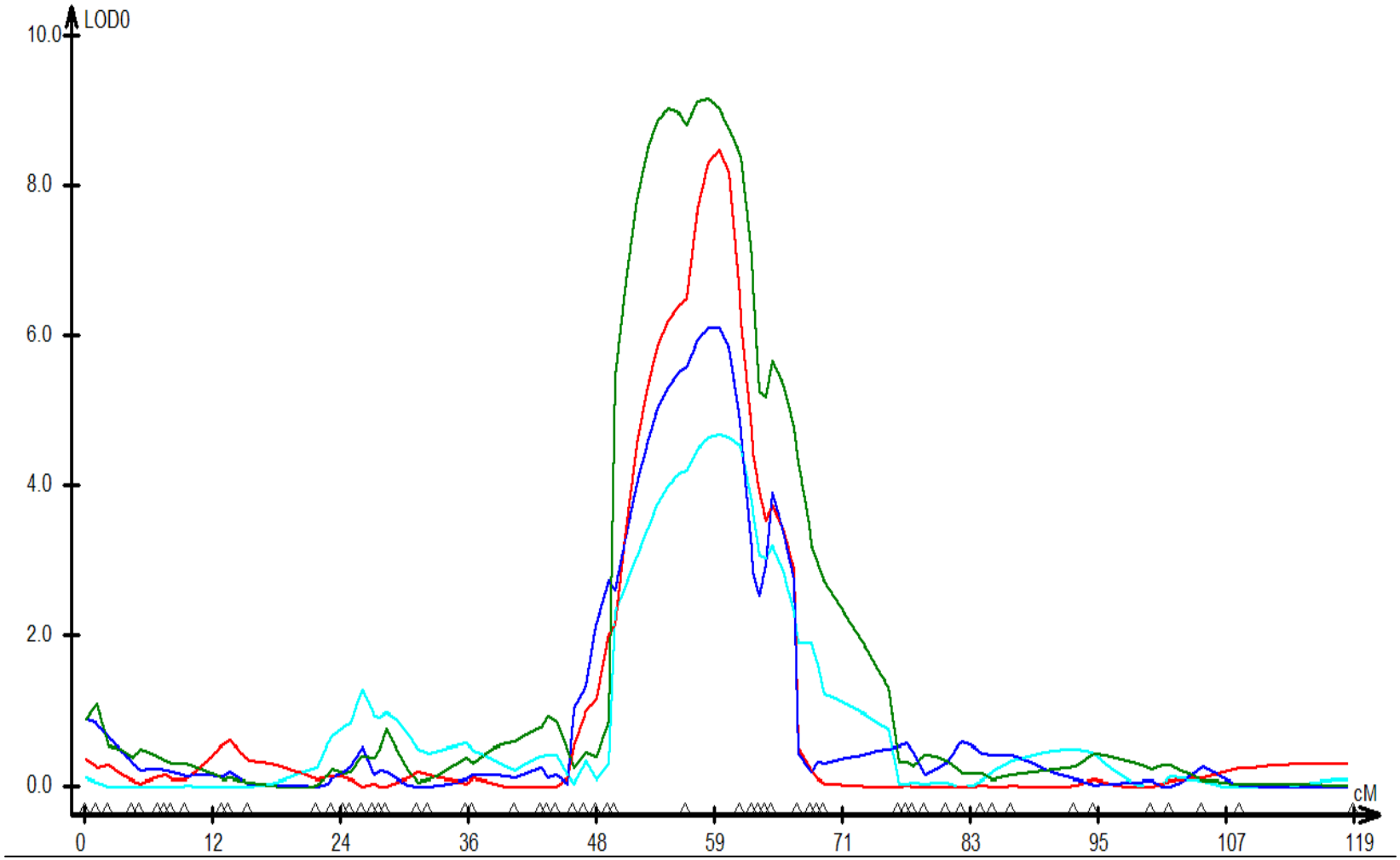


Figure 5.4. Quantitative trait loci (QTL) positions on LG-10 for flowering time in plants grown in greenhouse pots (green line) and in the field in 2008 (cyan line), 2009 (red line), and 2010 (blue line) to anthesis. LOD score is plotted as a function of marker position.

Discussion

Flowering Time

BTx642 flowered later than RTx7000 in both short day greenhouse conditions and field conditions. In 2009 and 2010 both genotypes flowered approximately six days sooner than they did in 2008 most likely due to the fact that average daily temperatures were approximately 5º C higher in June and July of 2009 compared to 2008 which probably sped up the development of the plants in 2009. In 2010 the population was planted earlier than in 2008; therefore day lengths were shorter in 2010 than 2008 when plants exited the juvenile phase, and this may have resulted in earlier flowering. The largest differences in flowering time were observed under 10 hour short day conditions in the greenhouse where flowering time in BTx642 occurred ~70 DAE, ~20 days after RTx7000. In contrast, in the field where day lengths increased from 12.5-13 hour days in April to 14 hour days in July, BTx642 flowered at 72 DAE, only 4 days later than RTx7000. The most rapid flowering was observed when plants were grown in the greenhouse under 10 hour days from mid January to the beginning of April. Previous studies have shown that flowering is accelerated when night temperatures are low (18-21º C) and day temperatures do not exceed ~28º C (Quinby, 1974), similar to growth conditions during this experiment. Overall, these results suggest that time to flowering in both genotypes is influenced to varying extents by growing conditions that affect the rate of plant growth including average temperature, day/night temperature and light conditions (intensity and duration). These effects are likely responsible for the high amount of environmental variation observed (45%). Day length was probably an

important factor in this difference as experiments were included that were done under both short day (10 hour) and field (day shortening to summer solstice, then lengthening) conditions. Broad sense heritability was 0.68 across these conditions.

The genetic loci that modulate variation in flowering time in BTx642 and RTx7000 were identified through analysis of a RIL population derived from these genotypes. Flowering time of the RILs was assayed in short days and in field conditions leading to the identification of four main QTL regions that modulate flowering time. All four QTL were detected using both composite interval mapping and multiple interval mapping, though not all QTL were detected in all environments. Multiple interval mapping also allowed for detection of QTL interactions which if present, would suggest association with common biochemical pathways. No interactions were detected at 95% significance.

Individual RILs in the population flowered earlier or later than either parent, consistent with transgressive segregation. The QTL on LG-01 centered at 14.3 cM was observed in short days, whereas the QTL on LG-01 centered at ~80 cM and on LG-06 were not detected under these conditions. The flowering time QTL located on LG-08 were detected under all conditions and likely contain at least two different genes that modulate flowering time. The flowering time QTL on LG-10 was detected in all conditions and explained up to ~39% of the phenotypic variation in long days. Detailed analysis of RIL genotypes and phenotypes delimited this QTL to a region spanning ~6.2 cM that encodes the sorghum homolog of *CONSTANS* (*CO* or *Hd1*), a CCT-domain protein involved in photoperiod sensing and flowering time regulation in Arabidopsis,

rice and other plants (Putterill et al., 1995). Further analysis will be required to verify that allelic variation in *CO* is the basis of this QTL. However, in rice, *CO* acts a repressor of flowering in long days consistent with the action of the BTx642 version of this QTL.

Stalk Length

Plant stalks are comprised of the stem and leaf sheaths. The stalk is important as a conduit for water and nutrients connecting roots, leaves and panicles and can be a significant sink for non structural carbohydrates that can be mobilized during grain filling (Borrell et al., 2000a). Variation in stalk length was examined in the BTx642 x RTx7000 RIL population at anthesis under greenhouse and field conditions. At anthesis, height to the flag leaf is mostly due to the length and number of internodes with upper internodes contributing more to overall stalk length. Broad sense heritability was high both across years in the field (0.95) and across locations (0.86). Environment played a much larger role in total variance across locations as opposed to across years, explaining 37.6% of the variance across locations and only 0.1% of the variance across years.

Overall, four to five QTL for stalk length were detected using composite interval mapping. All of these QTL were also detected by multiple interval mapping. Analysis of individual RIL genotypes and phenotypes delimited the large effect QTL on LG-06 to a region spanning ~872 Kbp (~2.5 cM) that aligned to the *Dw2* locus (Klein et al., 2008).

A stalk length QTL corresponding to the other known height locus, *Dw3*, located on chromosome 7 was not detected in the current studies. One likely explanation for not detecting this QTL is that BTx642 and RTx7000 have the same genotype spanning this

region of SBI-07. This is most likely due to the fact that both plants contain *dw3* derived from Blackhull kafir. RTx7000 (Caprock) has Blackhull kafir in its pedigree and is known to have the genotype *dw1Dw2dw3dw4* (Quinby, 1974). BTx642 was converted to an early flowering short genotype by crossing to BTx406, a four dwarf genotype. BTx406 contains the recessive form of *dw3* derived from BTx398 (Martin) that also has Blackhull kafir in its pedigree, the likely source of *dw3*.

Multiple interval mapping did not find any significant interactions at 95% confidence between QTL, suggesting that underlying genes may not be part of the same pathway. However, additional studies are required to confirm this result.

CHAPTER VI

GENETIC BASIS OF SHOOT WEIGHT VARIATION IN BTx642 AND RTx7000

Introduction

The sorghum life cycle can be divided into three main stages of development. Growth stage 1 (GS1) is comprised of a juvenile and a vegetative phase. Quinby (1967) determined that the juvenile phase in grain sorghum lasts a minimum of ten to fourteen days after emergence and that sorghum is photoperiod insensitive during this period of development. The photoperiod sensitive portion of growth stage 1 varies in length depending on photoperiod and other factors that modulate time to floral initiation. The beginning of growth stage 2 (GS2) is marked by initiation of the floral apical meristem and ends at anthesis. During GS2, leaves initiated prior to floral initiation expand to their full size, internode length increases ('booting'), and reproductive structures are formed and exerted above the upper most leaf and leaf sheath. Growth stage 3 (GS3) begins when sorghum flowers (anthesis) and ends at grain maturity. Grain filling occurs during GS3, a developmental phase that is typically 6-7 weeks in duration (Smith and Frederiksen, 2000).

Carbon partitioning refers to the distribution of photosynthate among plant organs where it is used for respiration or accumulated in a 'sink' either as structural carbohydrate (i.e., cellulose, hemicellulose, lignin) or as non-structural carbohydrate (starch, sugars). Carbon and nitrogen utilization and accumulation within the shoot varies depending on growth stage. In GS1 the seed and leaves act as the primary source

of carbohydrate and as a major sink due to the growth of leaves. Assimilate is also transported and used in other sinks such as the growing stem and root system. In GS2 the leaf continues to act as a source of photosynthate and as a sink while leaves are expanding. The stalk requires higher input of carbon during GS2 as internodes expand and reproductive structures are formed. In GS3, the panicle develops high sink strength requiring carbon and nitrogen from the leaf, stem, and root. Studies of the distribution of radiolabeled photosynthate from leaves show that in general the upper leaves serve as a source of assimilate for the shoot apical meristem, the middle leaves supply the stem, and the lower leaves supply both the stem and the root system (Palit, 1985). Additionally, in many species the reproductive organs acquire photosynthate predominantly from nearby leaves (Khan and Sagar, 1967; Wardlaw, 1968; Kriedemann, 1970).

Several studies have focused on factors that affect carbon partitioning and the impact of stresses such as nutrient deficit, high or low temperature, and drought on carbon partitioning. Environments with limiting nutrients can lead to an accumulation of photosynthate in leaves and stems as a result of stunted growth (Wardlaw, 1985). Low nitrogen levels have been shown to increase sink strength and dry weight in the roots (Rufty et al., 1984). Low phosphorus levels leading to lower grain yield have been correlated with lower export of assimilates from the flag leaf to the grain (Batten and Wardlaw, 1987). Similar to low nutrient levels, low temperature can alter carbon partitioning by causing retention of assimilate in leaves and stems, resulting in retarded growth (Farrar, 1988). Under drought stress carbon partitioning is affected by increasing

the concentration of water-soluble carbohydrates (osmoregulation) (Timpa et al., 1986). Although this helps in lowering the osmotic potential, carbon partitioning to grain can be hindered as source assimilate may be limiting due to decreased photosynthesis (Rawson and Evans, 1971).

In this chapter variation in shoot parameters, including leaf, stalk, panicle, and overall shoot weight were examined in a RIL population derived from BTx642 x RTx7000 under field and greenhouse conditions at anthesis. QTL analysis was used to help examine relationships between genetic pathways that modulate shoot biomass traits.

Results

The study of BTx642 and RTx7000 phenology in Chapter II identified differences between these genotypes in shoot, leaf, and stalk weight at various growth stages. Therefore, shoot, leaf, and stalk weight of the parental lines and the RIL population were analyzed at anthesis both in the field in 2009 and 2010 and in greenhouse conditions to determine if QTL modulating these traits could be identified. In all cases, the plants were grown under well-watered conditions.

Table 6.1 summarizes data on shoot biomass traits collected from BTx642, RTx7000 and the BTx642 x RTx7000 RIL population grown in various environments. At anthesis, BTx642 accumulated similar amounts of shoot biomass compared to RTx7000 under field conditions both in 2009 (346 g fw shoot and 82 g dw shoot for BTx642 vs. 386 g fw shoot and 87 g dw shoot for RTx7000) and 2010 (285 g fw shoot and 67 g dw shoot for BTx642 vs. 300 g fw shoot and 61 g dw shoot for RTx7000). However, under short day and low light greenhouse conditions BTx642 produced

considerably more shoot biomass than RTx7000 (81 g fw shoot and 15 g dw shoot for BTx642 vs. 25 g fw shoot and 4 g dw shoot for RTx7000). BTx642 accumulated approximately four times and RTx7000 approximately 14 times more biomass under field conditions relative to greenhouse pot grown plants. The RIL means for the population were within parental means when plants were grown both in the field in 2009 and in the greenhouse, and were above parental means when plants were grown in the field in 2010. At anthesis all effects were significant across years in the field (Table 6.2). The genetic contribution to total variance ranged from 35-69% of the total variance, while broad sense heritability ranged from 0.77-0.90. Across field and greenhouse environments, the genetic portion of total variance was only significant ($P<0.05$) with respect to leaf weight (Tables 6.2 and 6.3). Environmental effects were highly significant, ranging from 70-89% of the total variance. Broad sense heritability was low for all shoot biomass parameters, ranging from 0.12-0.37 across locations.

Table 6.1. Shoot biomass parameters for BTx642 x RTx7000 parental and recombinant inbred lines (RILs) grown in the field in 2009 and 2010 and in greenhouse conditions.

Trait	BTx642	RTx7000	RIL Population		
	Mean	Mean	Mean (SD[†])	Min.-Max.	CV%[‡]
Field 2009					
Shoot fresh weight (g)	345.95	386.23	365.31 (82.25)	215.10-582.67	22.5
Shoot dry weight (g)	81.50	86.78	87.53 (17.23)	53.17-134.68	19.7
Leaf fresh weight (g)	96.56	90.76	89.99 (24.65)	44.39-159.22	27.4
Leaf dry weight (g)	23.30	20.13	21.46 (6.06)	10.52-36.68	28.2
Stalk fresh weight (g)	213.00	228.31	213.45 (66.21)	91.90-374.51	31.0
Stalk dry weight (g)	46.73	44.70	44.52 (13.84)	17.60-80.47	31.1
Field 2010					
Shoot fresh weight (g)	284.84	299.54	312.22 (90.57)	164.54-727.73	29.0
Shoot dry weight (g)	66.92	61.39	67.32 (20.03)	37.51-152.07	29.8
Leaf fresh weight (g)	68.75	67.32	72.61 (22.91)	36.24-170.35	31.6
Leaf dry weight (g)	18.04	16.03	17.46 (5.49)	8.60-39.12	31.5
Leaf sheath fresh weight (g)	73.81	65.45	72.20 (18.91)	36.58-172.30	26.2
Leaf sheath dry weight (g)	16.00	12.41	14.41 (4.10)	7.39-34.67	28.4
Stalk fresh weight (g)	162.70	177.39	186.49 (69.55)	76.91-524.30	37.3
Stalk dry weight (g)	34.94	30.27	35.48 (14.11)	16.30-103.63	39.8
Stem fresh weight (g)	61.56	82.42	84.12 (47.31)	20.99-300.69	56.2
Stem dry weight (g)	14.04	13.49	16.15 (9.35)	4.62-60.96	57.9
Greenhouse					
Shoot fresh weight (g)	81.18	25.45	48.85 (17.77)	21.48-105.15	36.4
Shoot dry weight (g)	15.06	3.76	8.28 (3.39)	3.82-20.74	40.9
Leaf fresh weight (g)	24.93	7.78	14.02 (7.01)	4.94-37.81	50.0
Leaf dry weight (g)	5.23	1.31	2.70 (1.46)	1.03-8.34	54.0
Leaf sheath fresh weight (g)	24.29	7.24	13.33 (5.43)	4.98-33.97	40.7
Leaf sheath dry weight (g)	4.05	0.92	1.97 (0.90)	0.78-5.48	45.5
Stalk fresh weight (g)	42.75	14.95	26.95 (9.18)	12.66-56.39	34.1
Stalk dry weight (g)	7.26	1.90	4.04 (1.53)	1.96-9.71	37.9
Stem fresh weight (g)	9.86	5.26	8.67 (3.76)	2.02-18.47	43.4
Stem dry weight (g)	2.11	0.66	1.39 (0.59)	0.40-3.05	42.4

[†]Standard deviation.

[‡]Coefficient of variation.

Table 6.2. Analysis of variance across well-watered environments for shoot biomass parameters in the BTx642 x RTx7000 RIL population.

Trait[†]	Source	Across years in the field[‡]					Across locations[§]				
		df	MS[¶]		SS[#]	CoV[††]	TV%[‡‡]	df	MS[¶]		SS[#]	CoV[††]	TV%[‡‡]
LFW	Rep	1	3.40E+02		1.31E-03	9.31E-01	0.1	1	2.99E+01		7.22E-05	6.24E-02	<0.1
	G	89	2.01E+03	***	6.91E-01	4.43E+02	55.1	89	6.94E+02	*	1.49E-01	6.37E+01	3.2
	E	1	2.70E+04	***	1.04E-01	1.49E+02	18.6	1	3.06E+05	***	7.39E-01	1.71E+03	84.9
H^{YR} = 0.88	GxE	89	2.48E+02	*	8.50E-02	3.76E+01	4.7	89	4.41E+02	***	9.47E-02	2.01E+02	10.0
H^{LC} = 0.37	Error	178	1.73E+02		1.19E-01	1.73E+02	21.5	177	4.10E+01		1.75E-02	4.10E+01	2.0
LDW	Rep	1	5.55E+01		3.64E-03	2.52E-01	0.5	1	6.04E-03		2.36E-07	1.68E-02	<0.1
	G	89	1.17E+02	***	6.85E-01	2.53E+01	54.0	89	3.79E+01	*	1.32E-01	3.04E+00	2.4
	E	1	1.43E+03	***	9.38E-02	7.89E+00	16.8	1	1.94E+04	***	7.58E-01	1.09E+02	86.1
H^{YR} = 0.86	GxE	89	1.63E+01	**	9.53E-02	2.93E+00	6.3	89	2.58E+01	***	8.98E-02	1.15E+01	9.1
H^{LC} = 0.32	Error	178	1.05E+01		1.22E-01	1.05E+01	22.3	177	3.00E+00		2.08E-02	3.00E+00	2.4
STKFW	Rep	1	6.45E+01		3.44E-05	5.05E+00	0.1	1	1.16E+03		3.62E-04	4.57E+00	<0.1
	G	89	1.66E+04	***	7.90E-01	3.74E+03	68.5	89	5.23E+03		1.46E-01	1.68E+02	1.1
	E	1	6.54E+04	***	3.49E-02	3.56E+02	6.5	1	2.26E+06	***	7.08E-01	1.27E+04	82.8
H^{YR} = 0.90	GxE	89	1.74E+03	***	8.26E-02	3.87E+02	7.1	89	4.56E+03	***	1.27E-01	2.13E+03	13.9
H^{LC} = 0.13	Error	178	9.69E+02		9.20E-02	9.69E+02	17.8	177	3.45E+02		1.91E-02	3.45E+02	2.2
STKDW	Rep	1	6.06E+00		6.93E-05	3.04E-01	0.1	1	8.63E-02		6.81E-07	9.59E-02	<0.1
	G	89	6.74E+02	***	6.86E-01	1.43E+02	53.6	89	2.14E+02		1.50E-01	6.67E+00	1.1
	E	1	7.33E+03	***	8.38E-02	4.04E+01	15.2	1	8.80E+04	***	6.94E-01	4.93E+02	81.8
H^{YR} = 0.84	GxE	89	1.05E+02	***	1.07E-01	2.24E+01	8.4	89	1.87E+02	***	1.32E-01	8.58E+01	14.2
H^{LC} = 0.12	Error	178	6.04E+01		1.23E-01	6.04E+01	22.7	177	1.72E+01		2.40E-02	1.72E+01	2.8
SFW	Rep	1	1.04E+03		3.15E-04	6.96E+00	0.1	1	2.10E+03		2.69E-04	7.35E+00	<0.1
	G	89	2.63E+04	***	7.06E-01	5.71E+03	57.0	89	9.30E+03		1.06E-01	4.16E+02	1.1
	E	1	2.52E+05	***	7.62E-02	1.39E+03	13.9	1	6.17E+06	***	7.89E-01	3.46E+04	88.1
H^{YR} = 0.87	GxE	89	3.53E+03	**	9.47E-02	6.21E+02	6.2	89	7.64E+03	***	8.69E-02	3.45E+03	8.8
H^{LC} = 0.18	Error	178	2.29E+03		1.23E-01	2.29E+03	22.9	177	7.94E+02		1.80E-02	7.94E+02	2.0
SDW	Rep	1	1.28E+02		6.83E-04	1.45E-01	<0.1	1	4.24E-03		1.08E-08	2.78E-01	<0.1
	G	89	1.13E+03	***	5.36E-01	2.19E+02	34.8	89	4.52E+02		1.03E-01	2.07E+01	1.1
	E	1	3.66E+04	***	1.94E-01	2.03E+02	32.2	1	3.10E+05	***	7.91E-01	1.74E+03	88.3
H^{YR} = 0.77	GxE	89	2.60E+02	***	1.23E-01	5.29E+01	8.4	89	3.70E+02	***	8.39E-02	1.61E+02	8.2
H^{LC} = 0.18	Error	178	1.54E+02		1.46E-01	1.54E+02	24.5	177	4.95E+01		2.24E-02	4.95E+01	2.5

***Indicates significance at p<0.001, **p<0.01, or *p<0.05.

[†]L (leaf), STK (stalk), S (shoot), FW (fresh weight), DW (dry weight), H^{YR} (broad sense heritability across years), H^{LC} (broad sense heritability across locations).

[‡]2009 and 2010.

[§]Field 2010 and greenhouse.

[¶]Mean square.

[#]Proportion of total sums of squares.

[††]Covariate value.

[‡‡]Percentage of total variation.

Table 6.3. Analysis of variance across well-watered field 2010 and greenhouse conditions for stem and leaf sheath weight in the BTx642 x RTx7000 RIL population.

Trait	Source	df	MS[†]		SS[‡]	CoV[§]	TV%[¶]
	Rep	1	2.61E+02		2.81E-04	7.23E-01	<0.01
Stem FW	G	89	2.43E+03		2.33E-01	9.45E+01	2.3
	E	1	5.07E+05	***	5.45E-01	2.84E+03	70.3
H = 0.15	GxE	89	2.06E+03	***	1.97E-01	9.69E+02	24.0
	Error	177	1.32E+02		2.52E-02	1.32E+02	3.3
	Rep	1	1.20E-03		3.33E-08	3.52E-02	<0.01
Stem DW	G	89	9.31E+01		2.30E-01	2.83E+00	1.8
	E	1	1.94E+04	***	5.38E-01	1.09E+02	69.7
H = 0.12	GxE	89	8.18E+01	***	2.02E-01	3.81E+01	24.4
	Error	177	6.26E+00		3.07E-02	6.26E+00	4.0
Leaf	Rep	1	2.26E+02		6.05E-04	1.04E+00	<0.01
Sheath	G	89	4.21E+02		7.34E-02	1.89E+01	1.0
FW	E	1	3.08E+05	***	8.24E-01	1.73E+03	89.0
H = 0.18	GxE	89	3.46E+02	***	8.22E-02	1.54E+02	7.9
	Error	177	4.08E+01		1.93E-02	4.08E+01	2.1
Leaf	Rep	1	1.11E+00		6.48E-05	2.77E-03	<0.01
Sheath	G	89	1.92E+01		9.94E-02	8.63E-01	1.0
DW	E	1	1.38E+04	***	8.02E-01	7.73E+01	89.0
H = 0.18	GxE	89	1.58E+01	***	8.17E-02	7.13E+00	8.2
	Error	177	1.61E+00		1.66E-02	1.61E+00	1.8

[***] Indicates significance at the 0.001 probability level.

[†] Mean square.

[‡] Proportion of total sums of squares.

[§] Covariate value.

[¶] Percentage of total variation.

At grain maturity dry weight accumulation in the panicle was close to 1.5 times greater in RTx7000 relative to BTx642 in 2008 under field conditions and over 2 times greater in 2009 and 2010 (Table 6.4). The means of panicle weight for the RIL population were within parental means in all three years and were somewhat higher in 2008 than the other years. Coefficients of variation for panicle weight however, were consistent for all years. Variation of biomass traits among RILs was extensive, ranging up to 9-fold for selected traits. All effects were significant for panicle biomass, with genotype explaining 20% of total variation, environment 49%, and genotype by environment explaining 8% of total variation for panicle dry weight (Table 6.5). Broad sense heritability was 0.66 for panicle fresh weight and 0.68 for panicle dry weight.

Table 6.4. Panicle weight at grain maturity for BTx642 x RTx7000 parental and recombinant inbred lines (RILs) grown in field conditions in 2008-2010.

Trait	BTx642	RTx7000	RIL Population		
	Mean	Mean	Mean (SD†)	Min.-Max.	CV%‡
Field 2008					
Fresh weight (g)	63.57	79.08	78.51 (17.95)	32.67-124.72	22.9
Dry weight (g)	51.50	71.79	67.99 (16.92)	22.48-114.52	24.9
Field 2009					
Fresh weight (g)	54.93	102.01	76.10 (17.42)	29.38-116.95	22.9
Dry weight (g)	34.64	82.80	55.48 (14.06)	19.02-87.51	25.3
Field 2010					
Fresh weight (g)	28.10	57.25	48.19 (12.04)	15.12-91.60	25.0
Dry weight (g)	20.29	42.98	37.37 (9.90)	7.74-71.84	26.5

†Standard deviation.

‡Coefficient of variation.

Table 6.5. Analysis of variance across well-watered environments for panicle weight in the BTx642 x RTx7000 RIL population in 2008-2010.

Trait	Source	df	MS[†]		SS[‡]	CoV[§]	TV%[¶]
	Rep	1	4.84E+00		1.76E-05	5.11E-01	<0.1
FW	G	89	1.01E+03	***	3.26E-01	1.26E+02	20.7
	E	2	5.09E+04	***	3.70E-01	2.84E+02	46.5
H = 0.66	GxE	178	2.58E+02	***	1.67E-01	5.84E+01	9.6
	Error	267	1.42E+02		1.37E-01	1.42E+02	23.2
	Rep	1	1.57E-01		7.25E-07	4.02E-01	<0.1
DW	G	89	7.77E+02	***	3.19E-01	9.85E+01	20.3
	E	2	4.25E+04	***	3.92E-01	2.37E+02	48.9
H = 0.68	GxE	178	1.89E+02	***	1.55E-01	4.07E+01	8.4
	Error	267	1.08E+02		1.33E-01	1.08E+02	22.3

[***]Indicates significance at the 0.001 probability level.

[†]Mean square.

[‡]Proportion of total sums of squares.

[§]Covariate value.

[¶]Percentage of total variation.

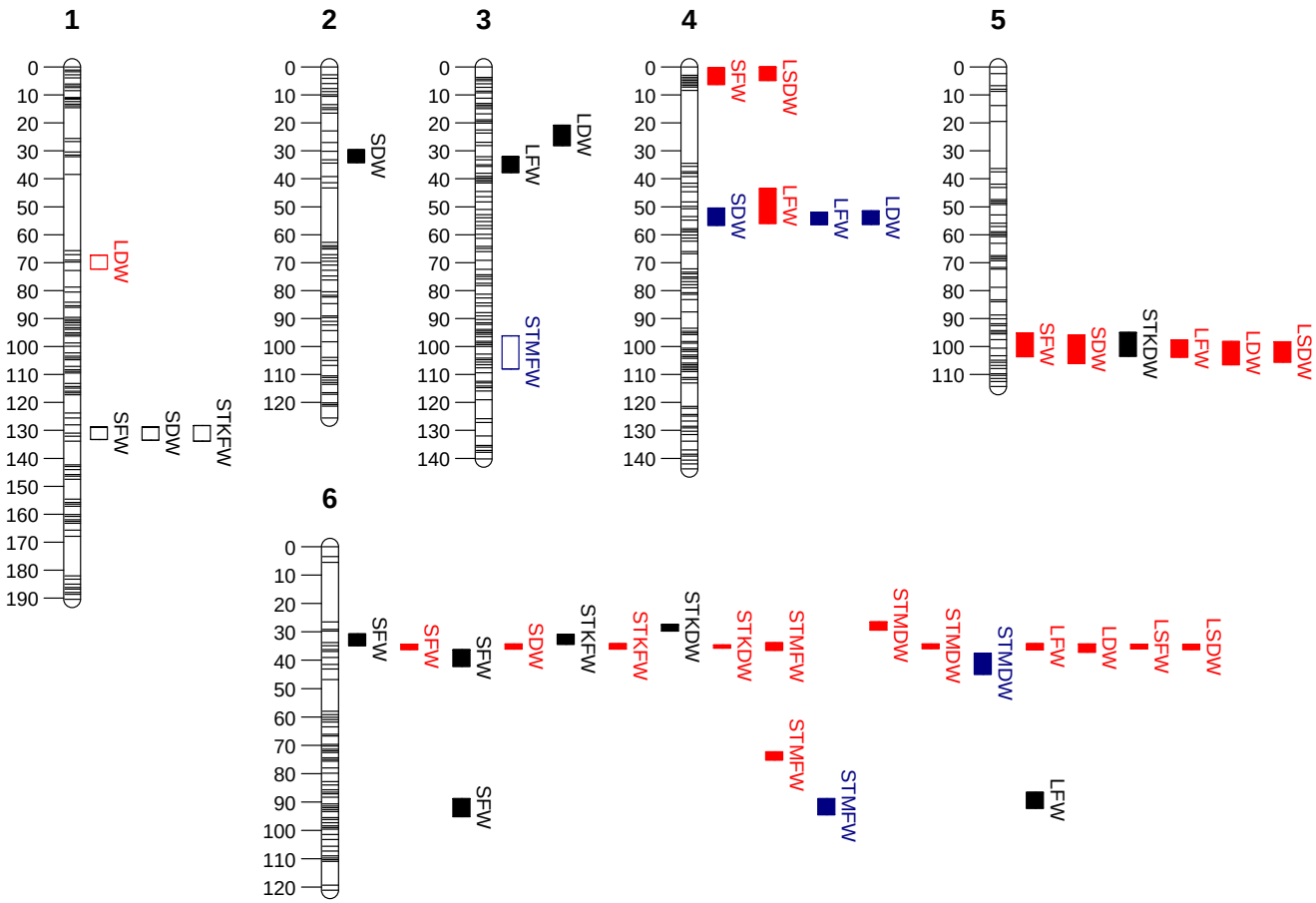


Figure 6.1. Quantitative trait loci (QTL) positions for shoot (S), leaf (L), stalk (STK), stem (STM), and leaf sheath (LS) fresh weight (FW) and dry weight (DW). Filled bars denote QTL where RTx7000 increased the magnitude of the trait; unfilled bars denote where BTx642 increased the magnitude of the trait. Field 2009 QTL are shown in black, field 2010 in red, and greenhouse in blue. All QTL are shown at one LOD below the QTL peak.

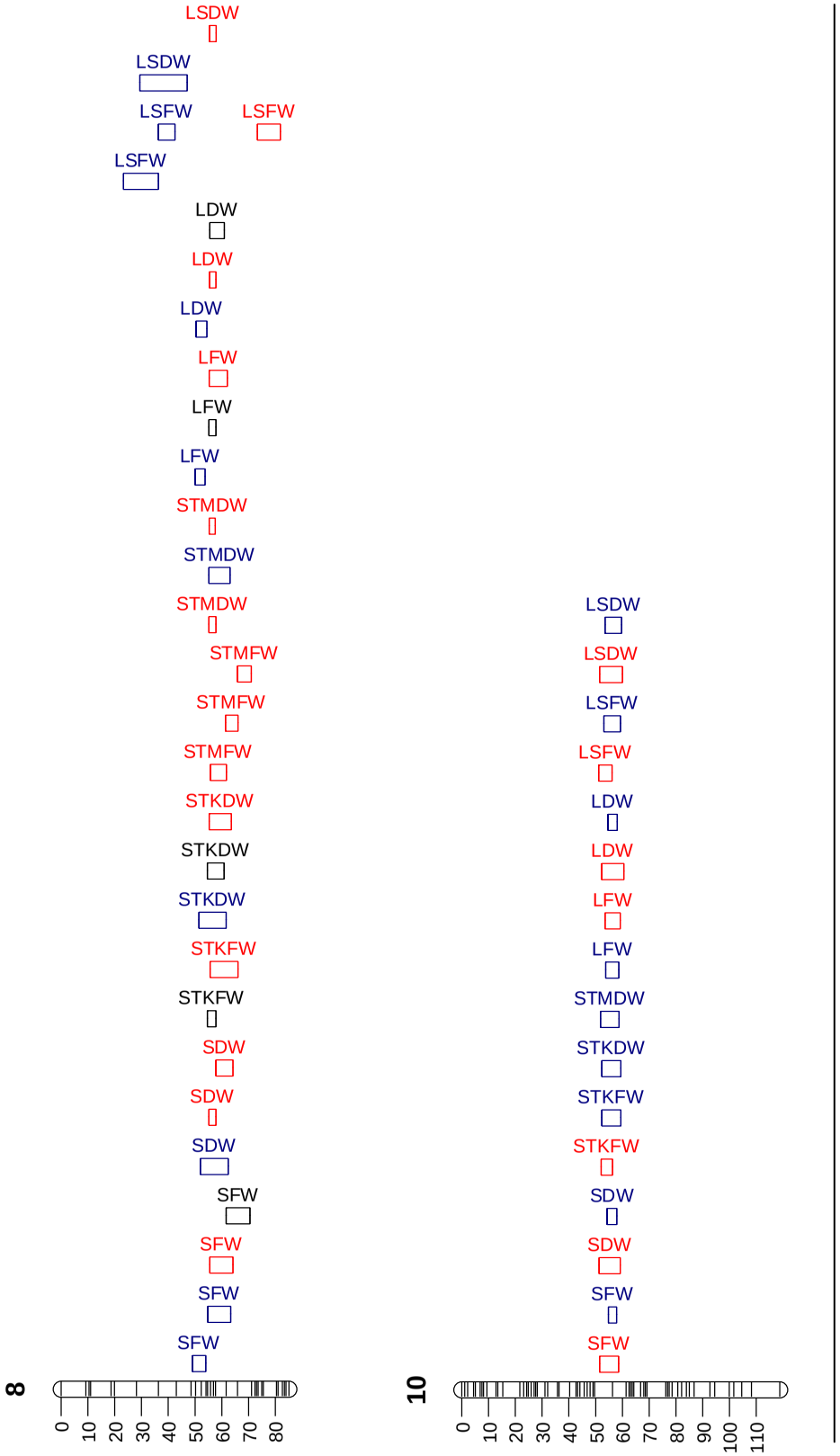


Figure 6.1, continued.

QTL Analysis

QTL analysis was conducted on the BTx642 x RTx7000 RIL population for shoot, leaf, and stalk biomass traits at anthesis field conditions in 2009 and 2010 and in greenhouse conditions. Only QTL with a LOD of 3.0 or greater determined by 1000 permutations at 95% confidence are described. As shown in Figure 6.1, QTL for shoot biomass traits were detected spanning 12 regions of the genome. In general, QTL identified in a given environment for shoot, leaf, and stalk biomass often co-localized in specific regions of the genome. QTL for shoot, leaf, and/or stalk weight for plants grown to anthesis in field conditions localized to LG-01, LG-02, LG-03, LG-04, LG-05, LG-06, LG-08, and LG-10, whereas plants grown in the greenhouse localized to LG-03, LG-04, LG-06, LG-08, and LG-10. In several cases, greenhouse and field-derived QTL co-localized (LG-04, LG-06, and LG-10).

In general, both BTx642 and RTx7000 alleles were associated mostly with high fresh and dry shoot, leaf, stalk, stem, and leaf sheath weights under field conditions (Table 6.1). In greenhouse conditions BTx642 alleles were associated with higher shoot biomass parameters relative to RTx7000. BTx642 alleles were responsible for increased biomass on LG-01, LG-03, LG-08, and LG-10 (Appendix B). RTx7000 alleles were responsible for increased biomass on LG-02, LG-03, LG-04, LG-05, LG-06, LG-08, and LG-10.

QTL for panicle weight were detected on LG-01, LG-02, and LG-05 (Fig. 6.2, Table 6.6). QTL were identified in unique areas of the genome each of the three years. BTx642 was responsible for increased panicle weight QTL detected on the beginning of

LG-02 and RTx7000 was responsible for increased panicle weight on LG-01, LG-05, and at the middle of LG-02.

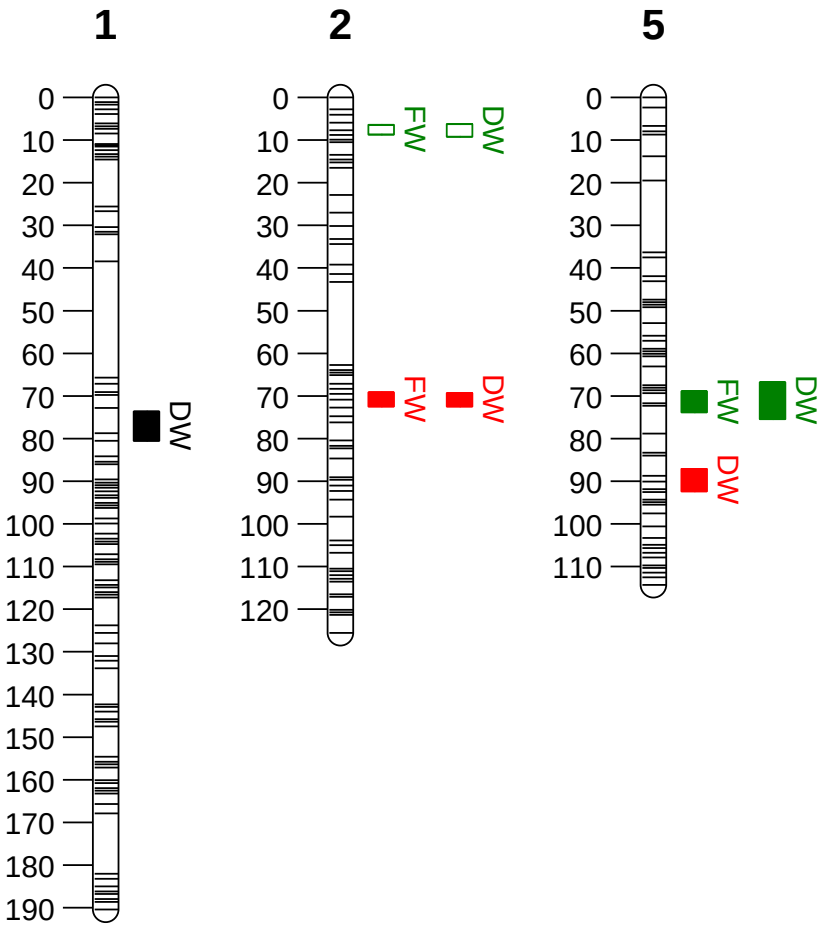


Figure 6.2. Quantitative trait loci (QTL) positions for panicle fresh weight (FW) and dry weight (DW) at grain maturity. Filled bars denote QTL where RTx7000 increased the magnitude of the trait; unfilled bars denote where BTx642 increased the magnitude of the trait. Field 2008 QTL are shown in green, 2009 in red, and 2010 in red. All QTL are shown at one LOD below the QTL peak.

Table 6.6. Panicle weight at grain maturity QTL for BTx642 x RTx7000 RILs in the field in 2008, 2009, and 2010.

Trait	Env.	LG[†]	Peak[‡]	LOD	LOD-1[§]	LOD-2[§]	a(H)[¶]	$R^{2\#}$	95% LOD
DW	2009	1	77.8	3.77	73.7 - 80.5	67.3 - 84.6	5.42	0.15	3.08
FW	2008	2	7.7	7.33	6.4 - 8.7	5.2 - 11.7	-8.65	0.21	3.24
DW	2008	2	7.7	6.42	6.3 - 9.2	4.9 - 11.9	-7.79	0.19	3.02
FW	2010	2	71.0	4.04	69.2 - 72.5	65.7 - 74.6	4.42	0.13	3.00
DW	2010	2	71.0	4.56	69.3 - 72.5	65.2 - 76.2	3.82	0.15	3.08
FW	2008	5	69.4	4.39	68.9 - 73.8	60.5 - 77.2	6.07	0.11	3.24
DW	2008	5	69.4	3.72	66.8 - 75.4	60.3 - 78.4	5.50	0.10	3.02
DW	2010	5	89.7	3.57	87.1 - 92.4	84.5 - 100.1	3.41	0.12	3.08

[†]Linkage group.

[‡]QTL peak in cM.

[§]QTL width in cM at either 1 (LOD-1) or 2 (LOD-2) LOD units below the peak.

[¶]Additive effect (days). Sign is with respect to RTx7000.

[#]Proportion of total phenotypic variance explained by the QTL.

Discussion

The accumulation of total plant biomass is determined in part by the extent of leaf area production that enables the capture and utilization of light energy for carbon fixation. The production of leaf area is under genetic control (see next chapter) and radiation use efficiency is related to the type of photosynthesis (C3, C4) carried out by the plant species being studied and other factors. In addition to plant development and time, the environment (climate, nutrients, and biotic factors) and intrinsic regulation modulate the rate and extent of biomass accumulation. Sink strength and biomass partitioning among organs (leaf, shoot, root, and panicle) may also impact biomass accumulation, assimilation of C and N, and final yield, especially in grain crops.

The current study was conducted to characterize the extent and genetic basis of variation in biomass accumulation and partitioning in a RIL population derived from BTx642 and RTx7000. Experiments were performed at anthesis and at grain maturity to identify QTL that modulate shoot, leaf, stalk/stem and panicle weight in the RIL population. Trait variation of the RILs was extensive when plants were grown in different environments. For example, at anthesis mean RIL shoot fresh weight was 312 g when plants were grown in the field in 2010 and 49 g under greenhouse conditions. Analysis of QTL using plants grown in different environments allowed for the identification of QTL that are important determinants of biomass in the environment analyzed and QTL of more general impact. Similarly, QTL that affect both leaf and stalk biomass accumulation and those that affect partitioning among these organs were detected using this experimental approach.

Variation in biomass accumulation among RILs in a given environment ranged from ~3-14 fold. Broad sense heritability ranged from 0.77-0.90 across years in the field and 0.12-0.37 across field and greenhouse locations. Across greenhouse and field environments, environmental effects composed >70% of the total variation, in essence making the genetic effect insignificant in all but two traits. Genetic effects are likely to become more significant with more reps (only two reps were used in the current study) and by using more advanced statistics to fix environmental effects while allowing genetic effects to be random. High biomass trait heritability across field conditions enabled the identification of approximately 12 QTL regions that modulate leaf, leaf sheath, stalk, stem, or panicle biomass at each stage of development analyzed. The

weight of BTx642 panicles at grain maturity was lower than RTx7000, consistent with previous studies (Rosenow and Clark, 1981; Sowder et al., 1997). Panicle weight and grain yield varied ~4-fold among RILs at grain maturity under field conditions. There was a general correlation between panicle weight and grain yield under the conditions analyzed, therefore panicle weight data was used for QTL analysis. Eight QTL were identified that modulated panicle weight at grain maturity. RTx7000 alleles increased panicle weight in six of the eight QTL, consistent with higher grain yield in RTx7000.

Several QTL were found to co-localize in discrete regions on LG-03, LG-06, LG-08, and LG-10 (Figure 6.1). Upon closer inspection, these regions align with QTL for flowering time. Chapter V identified significant QTL for flowering time on LG-03, LG-06, LG-08, and LG-10. Although QTL analysis alone can't definitively prove that the same allelic difference is responsible for two co-localizing QTL, other evidence can help support this hypothesis. First, the QTL for shoot biomass parameters and flowering time are shifted on LG-06 and LG-08 in the same pattern, depending on environment. Variance analysis showed that environmental and genotype by environment effects were significant across years in the field and locations, and these effects could contribute to the small shifts in QTL location observed. Importantly, within a given environment, flowering time and shoot weight parameters co-localize on the aforementioned linkage groups. Second, the co-localized regions have the same parent allele increasing the magnitude of the traits. Based on the phenology study, it is expected that plants which flower later have more internodes, grow taller, produce more leaves, have longer vegetative growth duration, and accumulate more biomass assuming no other

limitations. Therefore, the observed association between QTL/alleles that delay flowering time and QTL/alleles for increased leaf and shoot biomass accumulation is consistent with results from the phenology study and our general understanding of plant growth and development. It should be noted however that other regions of the genome unrelated to flowering time were identified that also modulate shoot biomass accumulation. The molecular basis of action of these alleles is currently unknown.

Shoot biomass traits have been analyzed previously in a Rio x BTx623 RIL population. Rio is a source of stay green from the caudatum race originating in Sudan, whereas BTx642 is a source of stay green from the durra race originating in Ethiopia. Murray et al. (2008) identified shoot, leaf, and stalk QTL for biomass on the end of LG-06 that were also identified in the current study of BTx642 and RTx7000. This correlation is most likely caused by the flowering time QTL corresponding to *Ma1* identified in both populations that maps in this region of the sorghum genome.

Panicle weight and grain yield can be modulated by many factors including total photosynthetic capacity, nitrogen supply, water deficit, and intrinsic factors that modulate sink strength. Panicle weight varied significantly among RILs derived from BTx642 and RTx7000 and QTL that modulate panicle weight at grain maturity were identified on LG-01, LG-02, and LG-05. No QTL were found overlapping QTL for shoot biomass parameters, flowering time, stalk length, or total leaf area (next chapter). This indicates that the genetic loci that modulate leaf and stalk biomass at anthesis are not directly determining the amount of panicle biomass that accumulates from anthesis to grain maturity in well-watered conditions.

CHAPTER VII

GENETIC BASIS OF LEAF SIZE VARIATION IN BTx642 AND RTx7000

Introduction

The leaf functions in the plant as a source of photosynthate and as a regulator of water loss and gas exchange for the plant. These functions are achieved through three primary tissues that make up the leaf: the epidermis, mesophyll, and vascular bundles. The epidermis forms the outer boundary of the leaf and serves as both a protective layer for underlying tissues and as a barrier to water loss. Water loss and gas exchange occur through pores in the epidermis called stomata. Stomatal aperture is regulated through bordering guard cell expansion and contraction. Mesophyll cells contain chlorophyll and the photosynthetic apparatus. The leaf contains palisade mesophyll that are tightly packed elongated cells running perpendicular to the epidermis, and spongy mesophyll composed of cells arranged loosely beneath the palisade mesophyll amongst large intracellular spaces that facilitate gas exchange. The third type of leaf tissue is the vascular bundle that runs parallel to the mesophyll cells and is composed of xylem and phloem tissues. The xylem facilitates water and mineral movement through the leaf while the phloem facilitates transport of sugars and other compounds. Vascular bundles in the leaves are connected to the vascular system of the stem and roots, allowing for movement of water, minerals, and photosynthate to areas of active growth and storage.

The leaf grows through cell division and cell expansion. In the grass leaf, cell division leading to leaf formation starts as the intercalary meristem divides in two, half of which gives rise to the leaf and the other half of which gives rise to the leaf sheath

(Sharman, 1945; Kaufman, 1959). All cells for a given leaf are derived from these two meristems. Growth of the leaf proceeds from the base, with the oldest cells located on the leaf tip. While cell division is important for overall leaf size, cell expansion is also important, accounting for cell volumes that are typically 20-40 times greater in the fully expanded leaf than at the start of leaf unfolding (Dale and Milthorpe, 1983). Cell expansion, or the increase in cell volume, is driven by turgor, P (Ray et al., 1972). Turgor is related to cell volume by the bulk modulus of elasticity, ε, which equals the change in turgor per change in cell volume multiplied by cell volume (Tyree and Jarvis, 1982). The change in cell volume over time is often expressed as the difference between turgor and a standard yield threshold term, Y, multiplied by a wall yielding coefficient (wall plastic extensibility), σ (Lockhart, 1985). To account for differences in water potential between the cell and its environment ($\Delta\psi$) and the conductivity of the cell membrane and cell wall (L_p), the change in cell volume per change in time can be further expressed as: $(L_p * \sigma/L_p + \sigma) * [\Delta\psi + (P - Y)]$ (Lockhart, 1985).

Plants have evolved several leaf-based mechanisms to cope with water-limiting conditions including control of transpiration through regulation of stomatal aperture. Total and the rate of plant water use can also be altered by modulating leaf area, demonstrated in a study in which the bulk of transpiration differences across diverse sorghum genotypes were due to differences in leaf area (Mortlock and Hammer, 1999). Leaf wilting, folding, erectness, and rolling are other mechanisms that alter transpiration and plant water use (Wilson et al., 1980; Ludlow and Bjorkman, 1984). These leaf movements also alter the amount of solar radiation intercepted by the leaf surface,

affecting leaf temperature and reducing water loss (O'Toole et al., 1979). Sorghum is known to produce a thick epicuticular wax on its leaves and leaf sheaths that reflect light, thus reducing leaf temperature and water loss (Johnson et al., 1983).

In this chapter variation in leaf length, width, and area were examined in a RIL population derived from BTx642 x RTx7000 grown in well-watered environments including field conditions in 2008, 2009, and 2010, and in greenhouse pots. QTL analyses revealed associations between traits and the location of genomic loci that modulate expression of these traits.

Results

Leaf length, leaf width, and leaf area were examined among the BTx642 x RTx7000 RIL population and parental lines BTx642 and RTx7000 in field conditions in 2008, 2009, and 2010, and in greenhouse conditions. Leaves nine through 14 were measured in all environments. As a general trend, RTx7000 had longer and wider leaves with greater surface area than BTx642 with respect to leaves ten through 14 grown in the field (Table 7.1). However, a larger difference between the parental lines was seen between leaf length than leaf width. The RIL population means for each leaf and leaf trait were approximately within the parental means. The coefficient of variation ranged from ~7% to ~18% for leaf length, ~8% to ~17% for leaf width, and ~13% to ~32% for leaf area.

Table 7.1. Leaf size parameters for BTx642 x RTx7000 parental and recombinant inbred lines (RILs) grown in the field in 2008, 2009, and 2010 and in greenhouse conditions.

Trait	Env.	BTx642	RTx7000	RIL Population		
		Mean	Mean	Mean (SD[†])	Min.-Max.	CV%[‡]
Leaf Length (cm)						
9	2008	62.7	59.5	65.5 (7.1)	46.7-78.1	10.8
	2009	71.7	75.2	70.4 (7.9)	47.8-84.5	11.2
	2010	71.6	65.1	66.5 (11.9)	42.3-90.8	17.9
	GH	60.7	30.3	43.0 (9.8)	26.4-66.9	22.9
10	2008	65.7	67.6	69.5 (6.1)	52.0-82.1	8.8
	2009	66.6	83.2	71.9 (6.4)	55.2-83.7	8.8
	2010	74.0	74.6	70.9 (10.4)	48.8-90.3	14.2
	GH	67.5	38.4	50.8 (8.5)	35.7-71.6	16.8
11	2008	65.0	71.3	70.9 (4.8)	58.9-83.2	6.8
	2009	61.4	78.1	72.1 (5.3)	61.8-83.2	7.3
	2010	73.7	83.0	74.0 (7.7)	53.2-88.9	10.5
	GH	71.1	46.5	56.7 (7.7)	42.6-73.3	13.5
12	2008	62.2	73.1	70.0 (4.9)	57.8-83.1	7.1
	2009	58.1	75.2	70.7 (5.7)	61.6-84.1	8.0
	2010	72.4	83.7	74.5 (6.3)	59.6-88.2	8.5
	GH	72.1	50.4	60.6 (6.5)	46.7-73.9	10.7
13	2008	57.7	69.1	65.5 (6.2)	53.6-85.5	9.5
	2009	56.7	68.3	65.1 (6.4)	51.9-85.7	9.8
	2010	69.7	77.3	70.2 (6.2)	55.5-84.4	8.8
	GH	69.0	50.1	60.0 (6.3)	42.4-73.9	10.5
14	2008	44.9	49.8	48.9 (7.1)	35.7-72.5	14.6
	2009	44.8	42.5	47.4 (6.1)	35.9-61.6	12.8
	2010	55.2	55.9	51.5 (5.9)	39.1-63.3	11.5
	GH	48.0	31.1	41.7 (5.9)	28.7-56.6	14.2
Leaf Width (cm)						
9	2008	7.7	6.7	7.2 (0.9)	5.4-9.5	12.5
	2009	10.5	10.0	9.2 (1.1)	6.4-11.2	12.4
	2010	9.6	9.2	8.8 (1.5)	5.5-12.6	17.3
	GH	3.2	1.3	1.9 (0.8)	0.7-4.1	43.8
10	2008	8.2	7.2	7.7 (0.8)	5.9-10.1	10.3
	2009	9.5	10.4	9.4 (0.9)	7.2-11.0	10.0
	2010	10.2	9.1	9.3 (1.4)	6.1-12.6	14.6
	GH	3.6	1.6	2.4 (0.8)	1.1-4.4	32.3

Table 7.1., continued.

Trait	Env.	BTx642	RTx7000	RIL Population		
		Mean	Mean	Mean (SD[†])	Min.-Max.	CV%[‡]
Leaf Width (cm)						
11	2008	8.0	8.4	8.2 (0.8)	6.4-10.5	9.6
	2009	8.9	9.7	9.4 (0.7)	7.5-11.7	7.8
	2010	9.5	10.2	9.9 (1.1)	7.4-12.9	10.9
	GH	4.2	2.3	3.0 (0.7)	1.7-5.2	22.3
12	2008	7.5	9.1	8.4 (0.8)	6.9-10.4	9.0
	2009	9.6	9.5	9.1 (0.8)	7.5-11.3	9.0
	2010	10.4	10.6	9.9 (1.1)	7.7-12.3	10.6
	GH	4.5	3.0	3.4 (0.6)	2.3-5.3	16.3
13	2008	7.5	9.2	7.9 (0.8)	6.0-10.0	9.7
	2009	7.0	7.8	8.4 (0.9)	6.8-10.2	10.6
	2010	10.6	10.0	9.5 (0.9)	7.7-12.0	9.8
	GH	4.8	3.1	3.6 (0.7)	2.7-5.7	18.4
14	2008	6.4	7.8	6.6 (0.7)	4.7-8.3	10.7
	2009	6.5	6.6	6.6 (0.8)	5.0-8.9	12.1
	2010	8.2	8.5	7.8 (0.9)	5.6-10.1	11.7
	GH	4.8	2.8	3.7 (0.7)	2.6-5.7	18.5
Leaf Area (cm^2)						
9	2008	331.1	275.7	333.2 (70.3)	168.9-506.1	21.1
	2009	410.3	451.1	392.4 (86.9)	189.4-573.5	22.1
	2010	385.0	317.6	337.5 (108.6)	119.1-603.3	32.2
	GH	127.8	23.3	56.5 (40.3)	12.0-188.2	71.4
10	2008	361.2	350.3	376.3 (60.3)	215.4-520.1	16.0
	2009	380.4	488.3	413.6 (68.5)	259.7-576.2	16.6
	2010	392.0	397.6	385.1 (94.3)	176.3-615.5	24.5
	GH	165.7	39.6	85.6 (43.3)	26.5-227.1	50.6
11	2008	354.3	411.7	401.0 (52.7)	258.5-545.4	13.1
	2009	327.5	468.2	415.3 (57.0)	275.0-593.0	13.7
	2010	399.1	465.1	419.9 (75.4)	237.7-619.7	18.0
	GH	210.0	71.9	120.8 (45.1)	48.5-285.1	37.3
12	2008	319.8	462.7	398.8 (55.1)	290.5-550.7	13.8
	2009	272.6	428.7	385.1 (58.9)	276.7-551.1	15.3
	2010	390.7	478.6	422.9 (63.3)	282.8-576.3	15.0
	GH	248.0	104.8	150.4 (45.0)	78.6-307.7	29.9

Table 7.1., continued.

Trait	Env.	BTx642	RTx7000	RIL Population		
		Mean	Mean	Mean (SD[†])	Min.-Max.	CV%[‡]
Leaf Area (cm^2)						
13	2008	274.8	430.6	347.6 (61.0)	242.1-530.9	17.6
	2009	222.1	317.6	309.4 (55.6)	210.7-467.9	18.0
	2010	353.1	427.1	372.3 (57.9)	250.6-514.8	15.6
	GH	252.1	107.2	160.5 (45.0)	85.3-302.8	29.9
14	2008	160.1	252.3	199.2 (48.8)	100.6-346.9	24.5
	2009	114.4	141.3	158.1 (35.0)	93.4-269.0	22.2
	2010	214.4	233.3	213.0 (43.9)	125.6-307.8	20.6
	GH	159.5	57.4	104.2 (35.8)	43.8-218.0	34.4
TLA	2008	2088.5	2381.9	2336.7 (382.0)	1516.7-3348.4	16.3
	2009	2141.5	2679.4	2425.2 (359.7)	1618.5-3513.0	14.8
	2010	2471.3	2558.5	2430.8 (483.8)	1421.6-3666.9	19.9
	GH	1163.2	404.2	678.1 (245.8)	302.2-1502.6	36.2

[†]Standard deviation.

[‡]Coefficient of variation.

In greenhouse conditions, leaf size parameter trends between the parents were opposite of those seen in the field, with BTx642 possessing larger leaves. This trend was likely contributed by the delayed flowering observed in BTx642 (Chapter II). The RIL population means for each leaf and leaf trait were within parental means. Coefficients of variation were extensive for leaf size traits in the greenhouse: ~11-23% for leaf length, ~16-44% for leaf width, and ~30-71% for leaf area.

Across years in the field all effects were significant for most traits (Table 7.2). The genetic contribution to total variance ranged from ~32-60% for leaf length, ~13-29% for leaf width, and ~25-62% for leaf area. Broad sense heritability was 0.74-0.86 for leaf length, 0.55-0.80 for leaf width, and 0.66-0.86 for leaf area. Across field and greenhouse conditions, all effects were significant with environment contributing the most to total variation. Broad sense heritability ranged from 0.53 to 0.76 for leaf length, 0.47 to 0.57 for leaf width, and 0.33 to 0.60 for leaf area.

Table 7.2. Analysis of variance across well-watered environments for leaf size parameters in the BTx642 x RTx7000 RIL population.

Trait[†]	Source	Across years in the field[‡]						Across locations[§]					
		df	MS[¶]		SS[#]	CoV[††]	TV%[‡‡]	df	MS[¶]		SS[#]	CoV[††]	TV%[‡‡]
9L	Rep	1	4.57E+00		8.59E-05	6.47E-02	<0.1	1	4.41E+01		4.62E-04	9.87E-02	<0.1
	G	89	4.11E+02	***	6.89E-01	6.12E+01	59.8	89	3.17E+02	***	2.96E-01	4.21E+01	10.4
	E	2	1.16E+03	***	4.36E-02	6.26E+00	6.1	1	4.91E+04	***	5.14E-01	2.75E+02	67.7
H^{YR}=0.86	GxE	177	4.76E+01	***	1.58E-01	1.29E+01	12.6	89	1.50E+02	***	1.40E-01	6.23E+01	15.3
H^{LC}=0.53	Error	266	2.18E+01		1.09E-01	2.18E+01	21.4	177	2.65E+01		4.92E-02	2.65E+01	6.5
10L	Rep	1	3.32E+00		9.13E-05	4.64E-02	<0.1	1	5.49E+01		7.82E-04	1.95E-01	<0.1
	G	89	2.90E+02	***	7.09E-01	4.31E+01	62.6	89	2.46E+02	***	3.11E-01	3.78E+01	12.6
	E	2	2.34E+02	***	1.29E-02	1.13E+00	1.6	1	3.62E+04	***	5.16E-01	2.03E+02	67.8
H^{YR}=0.86	GxE	177	3.34E+01	***	1.63E-01	8.88E+00	12.9	89	9.55E+01	***	1.21E-01	3.79E+01	12.7
H^{LC}=0.61	Error	266	1.57E+01		1.15E-01	1.57E+01	22.8	177	2.02E+01		5.09E-02	2.02E+01	6.8
11L	Rep	1	9.44E-02		3.97E-06	4.69E-02	0.1	1	6.66E+00		1.33E-04	4.32E-02	<0.1
	G	89	1.67E+02	***	6.24E-01	2.35E+01	51.7	89	1.84E+02	***	3.28E-01	3.39E+01	15.7
	E	2	4.05E+02	***	3.41E-02	2.13E+00	4.7	1	2.66E+04	***	5.33E-01	1.49E+02	69.3
H^{YR}=0.81	GxE	177	2.69E+01	***	2.01E-01	7.20E+00	15.8	89	4.97E+01	***	8.84E-02	1.78E+01	8.3
H^{LC}=0.73	Error	266	1.26E+01		1.41E-01	1.26E+01	27.7	177	1.43E+01		5.08E-02	1.43E+01	6.7
12L	Rep	1	1.58E+01		6.86E-04	4.70E-03	<0.1	1	3.68E+00		1.08E-04	5.70E-02	<0.1
	G	89	1.33E+02	***	5.14E-01	1.74E+01	38.5	89	1.31E+02	***	3.43E-01	2.50E+01	17.4
	E	2	1.03E+03	***	8.92E-02	5.62E+00	12.4	1	1.71E+04	***	5.02E-01	9.57E+01	66.6
H^{YR}=0.74	GxE	177	2.97E+01	***	2.28E-01	7.63E+00	16.9	89	3.18E+01	***	8.32E-02	9.05E+00	6.3
H^{LC}=0.76	Error	266	1.46E+01		1.68E-01	1.46E+01	32.2	177	1.38E+01		7.20E-02	1.38E+01	9.6
13L	Rep	1	6.49E+01		2.19E-03	1.58E-01	0.3	1	1.18E+01		4.45E-04	4.04E-02	<0.1
	G	89	1.60E+02	***	4.79E-01	2.07E+01	35.5	89	1.18E+02	***	3.98E-01	2.08E+01	20.6
	E	2	1.46E+03	***	9.83E-02	8.00E+00	13.7	1	9.38E+03	***	3.55E-01	5.25E+01	52.2
H^{YR}=0.74	GxE	177	3.64E+01	***	2.17E-01	6.93E+00	11.9	89	3.56E+01	***	1.20E-01	8.38E+00	8.3
H^{LC}=0.70	Error	266	2.26E+01		2.03E-01	2.26E+01	38.7	177	1.90E+01		1.27E-01	1.90E+01	18.8
14L	Rep	1	9.08E+01		2.74E-03	1.99E-01	0.3	1	1.62E+00		6.32E-05	1.42E-01	0.1
	G	89	1.62E+02	***	4.35E-01	2.06E+01	32.3	89	9.61E+01	***	3.34E-01	1.39E+01	14.4
	E	2	7.89E+02	***	4.76E-02	4.22E+00	6.6	1	8.62E+03	***	3.37E-01	4.82E+01	50.1
H^{YR}=0.75	GxE	177	3.98E+01		2.13E-01	1.09E+00	1.7	89	4.10E+01	***	1.42E-01	7.04E+00	7.3
H^{LC}=0.58	Error	266	3.77E+01		3.02E-01	3.77E+01	59.1	177	2.70E+01		1.87E-01	2.70E+01	28.0
9W	Rep	1	1.02E+01	***	7.49E-03	3.53E-02	1.2	1	3.22E-01		6.63E-05	7.10E-04	<0.1
	G	89	6.30E+00	***	4.13E-01	8.46E-01	29.1	89	3.85E+00	**	7.06E-02	4.56E-01	1.8
	E	2	1.80E+02	***	2.65E-01	1.01E+00	34.6	1	4.25E+03	***	8.76E-01	2.38E+01	93.3
H^{YR}=0.77	GxE	177	1.27E+00	***	1.66E-01	2.58E-01	8.9	89	2.04E+00	***	3.74E-02	8.02E-01	3.1
H^{LC}=0.47	Error	266	7.60E-01		1.49E-01	7.60E-01	26.2	177	4.47E-01		1.63E-02	4.48E-01	1.8
10W	Rep	1	1.04E+01	***	9.44E-03	3.61E-02	1.5	1	5.51E-03		1.16E-06	2.53E-03	<0.1
	G	89	4.50E+00	***	3.64E-01	5.73E-01	24.3	89	3.34E+00	***	6.28E-02	4.55E-01	1.8
	E	2	1.49E+02	***	2.70E-01	8.31E-01	35.2	1	4.23E+03	***	8.91E-01	2.37E+01	94.2
H^{YR}=0.73	GxE	177	1.10E+00	**	1.76E-01	1.76E-01	7.5	89	1.54E+00	***	2.89E-02	5.46E-01	2.2
H^{LC}=0.54	Error	266	7.46E-01		1.80E-01	7.46E-01	31.6	177	4.56E-01		1.70E-02	4.57E-01	1.8

Table 7.2, continued.

Trait[†]	Source	Across years in the field[‡]						Across locations[§]					
		df	MS[¶]		SS[#]	CoV[††]	TV%[‡‡]	df	MS[¶]		SS[#]	CoV[††]	TV%[‡‡]
	Rep	1	1.07E+01	***	1.25E-02	3.77E-02	2.0	1	1.58E-01		3.46E-05	1.26E-03	<0.1
11W	G	89	3.32E+00	***	3.45E-01	4.47E-01	24.0	89	2.21E+00	***	4.31E-02	3.18E-01	1.3
	E	2	1.27E+02	***	2.97E-01	7.12E-01	38.2	1	4.21E+03	***	9.24E-01	2.37E+01	96.0
H^{YR}=0.80	GxE	177	6.63E-01		1.37E-01	1.97E-03	0.1	89	9.46E-01	***	1.85E-02	2.84E-01	1.2
H^{LC}=0.57	Error	266	6.67E-01		2.07E-01	6.67E-01	35.8	177	3.82E-01		1.48E-02	3.82E-01	1.5
	Rep	1	1.14E+01	***	1.40E-02	4.03E-02	2.3	1	4.05E-03		1.00E-06	1.93E-03	<0.1
12W	G	89	2.91E+00	***	3.18E-01	3.40E-01	19.5	89	1.86E+00	***	4.10E-02	2.33E-01	1.1
	E	2	1.06E+02	***	2.60E-01	5.90E-01	33.8	1	3.74E+03	***	9.23E-01	2.10E+01	96.0
H^{YR}=0.67	GxE	177	8.89E-01	*	1.93E-01	1.16E-01	6.7	89	9.38E-01	***	2.06E-02	2.97E-01	1.4
H^{LC}=0.50	Error	266	6.57E-01		2.15E-01	6.57E-01	37.7	177	3.48E-01		1.52E-02	3.48E-01	1.6
	Rep	1	2.48E+01	***	2.74E-02	8.96E-02	4.5	1	6.24E-01		1.84E-04	9.41E-04	<0.1
13W	G	89	2.47E+00	***	2.43E-01	2.49E-01	12.6	89	1.81E+00	***	4.76E-02	2.69E-01	1.5
	E	2	1.24E+02	***	2.73E-01	6.91E-01	35.0	1	3.08E+03	***	9.09E-01	1.73E+01	95.2
H^{YR}=0.59	GxE	177	9.93E-01		1.94E-01	5.07E-02	2.6	89	7.46E-01	**	1.96E-02	1.46E-01	0.8
H^{LC}=0.59	Error	266	8.93E-01		2.62E-01	8.93E-01	45.2	177	4.56E-01		2.38E-02	4.56E-01	2.5
	Rep	1	4.42E+00	*	6.11E-03	1.38E-02	0.9	1	1.94E-01		1.04E-04	1.72E-03	<0.1
14W	G	89	2.12E+00	***	2.60E-01	2.02E-01	13.3	89	1.78E+00	***	8.52E-02	2.57E-01	2.7
	E	2	8.59E+01	***	2.37E-01	4.78E-01	31.4	1	1.54E+03	***	8.31E-01	8.67E+00	90.7
H^{YR}=0.55	GxE	177	9.14E-01		2.23E-01	8.54E-02	5.6	89	7.61E-01	***	3.64E-02	1.32E-01	1.4
H^{LC}=0.57	Error	266	7.44E-01		2.73E-01	7.44E-01	48.8	177	4.99E-01		4.75E-02	4.99E-01	5.2
	Rep	1	2.74E+02		5.27E-05	6.17E+00	<0.1	1	3.10E+02		3.24E-05	4.16E+00	<0.1
9A	G	89	3.97E+04	***	6.80E-01	5.95E+03	58.6	89	1.59E+04	*	1.47E-01	1.32E+03	2.8
	E	2	1.91E+05	***	7.35E-02	1.05E+03	10.4	1	7.03E+06	***	7.34E-01	3.94E+04	84.6
H^{YR}=0.86	GxE	177	4.36E+03	***	1.49E-01	1.23E+03	12.1	89	1.06E+04	***	9.88E-02	4.82E+03	10.3
H^{LC}=0.33	Error	266	1.92E+03		9.83E-02	1.92E+03	18.9	177	1.05E+03		1.94E-02	1.05E+03	2.3
	Rep	1	3.94E+03		1.09E-03	8.97E+00	0.1	1	8.75E+01		8.71E-06	4.69E+00	<0.1
10A	G	89	2.84E+04	***	7.03E-01	4.28E+03	62.1	89	1.34E+04	**	1.19E-01	1.39E+03	2.7
	E	2	6.58E+04	***	3.66E-02	3.54E+02	5.1	1	7.99E+06	***	7.95E-01	4.48E+04	88.5
H^{YR}=0.88	GxE	177	2.96E+03	***	1.46E-01	7.12E+02	10.3	89	7.88E+03	***	6.98E-02	3.50E+03	6.9
H^{LC}=0.41	Error	266	1.54E+03		1.14E-01	1.54E+03	22.3	177	9.23E+02		1.63E-02	9.23E+02	1.8
	Rep	1	5.96E+03	*	7.61E-03	1.79E+01	0.4	1	6.54E+01		6.93E-06	3.65E+00	<0.1
11A	G	89	1.84E+04	***	2.93E-05	2.69E+03	58.5	89	1.02E+04	***	9.60E-02	1.31E+03	2.7
	E	2	1.65E+04	**	4.21E-02	7.92E+01	1.7	1	7.96E+06	***	8.44E-01	4.47E+04	91.5
H^{YR}=0.84	GxE	177	2.44E+03	***	5.51E-01	6.34E+02	13.8	89	4.97E+03	***	4.69E-02	2.14E+03	4.4
H^{LC}=0.51	Error	266	1.18E+03		4.00E-01	1.18E+03	25.6	177	7.15E+02		1.34E-02	7.15E+02	1.5
	Rep	1	9.01E+03	*	3.78E-03	2.82E+01	0.6	1	7.08E+02		9.10E-05	1.96E-01	<0.1
12A	G	89	1.43E+04	***	5.36E-01	1.87E+03	40.5	89	8.36E+03	***	9.57E-02	1.24E+03	3.1
	E	2	6.52E+04	***	5.47E-02	3.49E+02	7.6	1	6.61E+06	***	8.50E-01	3.71E+04	91.8
H^{YR}=0.73	GxE	177	3.25E+03	***	2.41E-01	8.93E+02	19.4	89	3.43E+03	***	3.93E-02	1.39E+03	3.4
H^{LC}=0.59	Error	266	1.47E+03		1.64E-01	1.47E+03	31.9	177	6.73E+02		1.53E-02	6.73E+02	1.7

Table 7.2, continued.

Trait[†]	Source	Across years in the field[‡]					Across locations[§]				
		df	MS[¶]		SS[#]	CoV[††]	TV%[‡‡]	df	MS[¶]		SS[#]	CoV[††]	TV%[‡‡]
13A	Rep	1	3.42E+03		1.30E-03	6.22E+00	0.1	1	5.59E+01		1.09E-05	5.28E+00	<0.1
	G	89	1.28E+04	***	4.34E-01	1.52E+03	29.0	89	7.98E+03	***	1.38E-01	1.25E+03	4.9
	E	2	1.79E+05	***	1.37E-01	9.87E+02	18.8	1	3.99E+06	***	7.75E-01	2.24E+04	87.3
H^{YR}=0.66	GxE	177	3.70E+03	***	2.50E-01	9.76E+02	18.6	89	3.03E+03	***	5.24E-02	1.02E+03	4.0
H^{LC}=0.60	Error	266	1.76E+03		1.78E-01	1.76E+03	33.4	177	9.96E+02		3.42E-02	9.96E+02	3.9
14A	Rep	1	1.92E+02		1.22E-04	3.72E+00	0.1	1	4.75E+02		2.69E-04	2.29E+00	<0.1
	G	89	6.88E+03	***	3.89E-01	8.19E+02	25.3	89	4.44E+03	***	2.24E-01	6.66E+02	8.4
	E	2	1.45E+05	***	1.84E-01	8.06E+02	25.0	1	1.05E+06	***	5.97E-01	5.91E+03	74.6
H^{YR}=0.67	GxE	177	2.02E+03	***	2.27E-01	4.18E+02	12.9	89	1.80E+03	***	9.06E-02	4.62E+02	5.8
H^{LC}=0.60	Error	266	1.18E+03		2.00E-01	1.18E+03	36.7	177	8.83E+02		8.85E-02	8.83E+02	11.1
TLA	Rep	1	4.36E+04		4.27E-04	8.64E+00	<0.1	1	1.07E+03		3.25E-06	1.04E+02	<0.1
	G	89	8.58E+05	***	7.48E-01	1.31E+05	68.0	89	3.91E+05	***	1.06E-01	5.10E+04	3.0
	E	2	4.70E+05	**	9.21E-03	2.21E+03	1.1	1	2.73E+08	***	8.32E-01	1.54E+06	90.8
H^{YR}=0.89	GxE	177	7.79E+04	***	1.35E-01	1.84E+04	9.5	89	1.89E+05	***	5.12E-02	8.52E+04	5.0
H^{LC}=0.52	Error	266	4.13E+04		1.08E-01	4.13E+04	21.4	177	1.96E+04		1.06E-02	1.96E+04	1.2

***Indicates significance at p<0.001, **p<0.01, or *p<0.05.

[†]Leaf number followed by L (length), W (width), or A (area). TLA (total leaf area), H^{YR} (broad sense heritability across years), H^{LC} (broad sense heritability across locations).

[‡]2008, 2009, and 2010.

[§]Field 2010 and greenhouse.

[¶]Mean square.

[#]Proportion of total sums of squares.

[††]Covariate value.

[‡‡]Percentage of total variation.

Total leaf area was measured for the RIL population and parental lines under all environmental conditions (Table 7.1). Under field conditions in 2008-2010, total leaf area was consistently higher in RTx7000; in greenhouse conditions BTx642 total leaf area was roughly 3-fold greater than RTx7000. For the RIL population, total leaf area was smallest in greenhouse pots with a mean of 678 ± 246 cm^2 and was largest under field conditions in 2010 with a mean of 2431 ± 439 cm^2. The coefficient of variation was similar across field environments, ranging from 14.8% to 19.9%; in greenhouse pots

the coefficient of variation was 36.2%. Broad sense heritability was high under field conditions (0.89) and lower across locations (0.52) (Table 7.2).

QTL Analysis

QTL analysis was conducted on leaf parameters of plants grown in all environments. QTL were detected in 14 major regions of the genome (labeled L1-L14) and spanning all linkage groups except seven and nine (Fig. 7.1, Appendix B). Additional QTL were detected not spanning the major regions and are also presented in Figure 7.1. LOD thresholds at the 95% confidence level for leaf length, width, and area QTL ranged from 2.97 to 3.27 (Appendix B). Individual QTL significance ranged from LOD 3.13 to 18.95 and on average explained from 4% to 59% of the phenotypic variance.

Twelve out of 14 of the major regions contained QTL that affected all leaf size parameters. The remaining two regions, L5 and L7, affected only leaf width and leaf area. Leaf number cohorts of leaf size parameters were observed across the genome. Five regions (L3, 5, 7, 9, and 10) contained QTL co-localizing for leaves 9-12 leaf size parameters, three regions (L2, 6 and 8) contained QTL for leaves 12-14 leaf size parameters, and in the remaining regions (L1, 4, and 11-14), QTL were identified for leaf size parameters of all leaves. Several QTL regions were environment-specific. QTL at regions L4, 5, 8, and 9-12 were detected in field conditions only, whereas L6 was detected only under greenhouse conditions. Interestingly, leaf size parameter QTL in greenhouse and field conditions were found co-localized in 6 out of the 14 regions (L1, 2, 3, 7, 13, and 14).

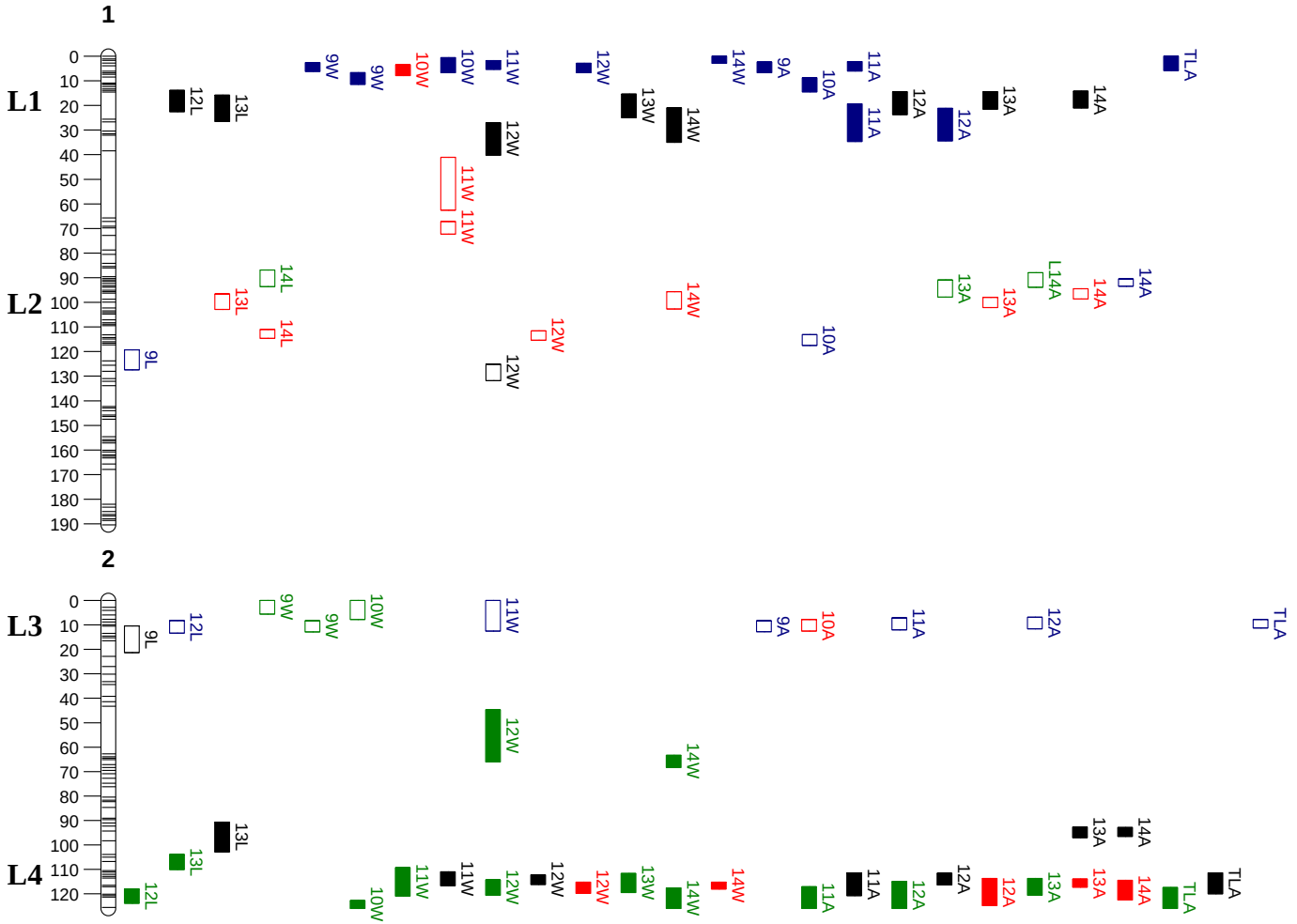


Figure 7.1. Quantitative trait loci (QTL) positions for leaves 9-14 leaf length (L), width (W), area (A), and total leaf area (TLA). Filled bars denote QTL where RTx7000 increased the magnitude of the trait; unfilled bars denote where BTx642 increased the magnitude of the trait. Field 2008 QTL are shown in green, field 2009 in black, field 2010 in red, and greenhouse in blue. All QTL are shown at one LOD below the QTL peak.

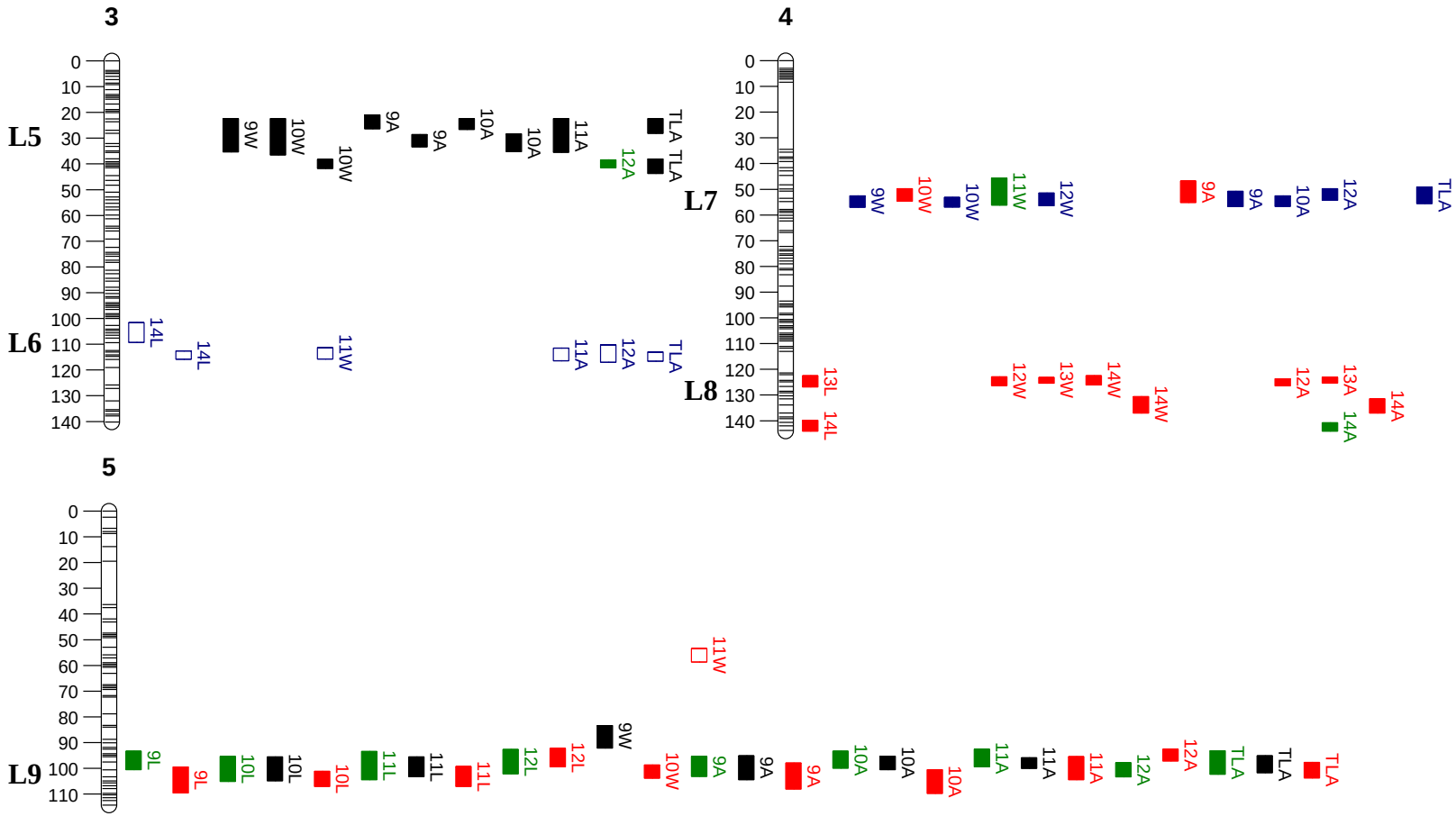


Figure 7.1, continued.

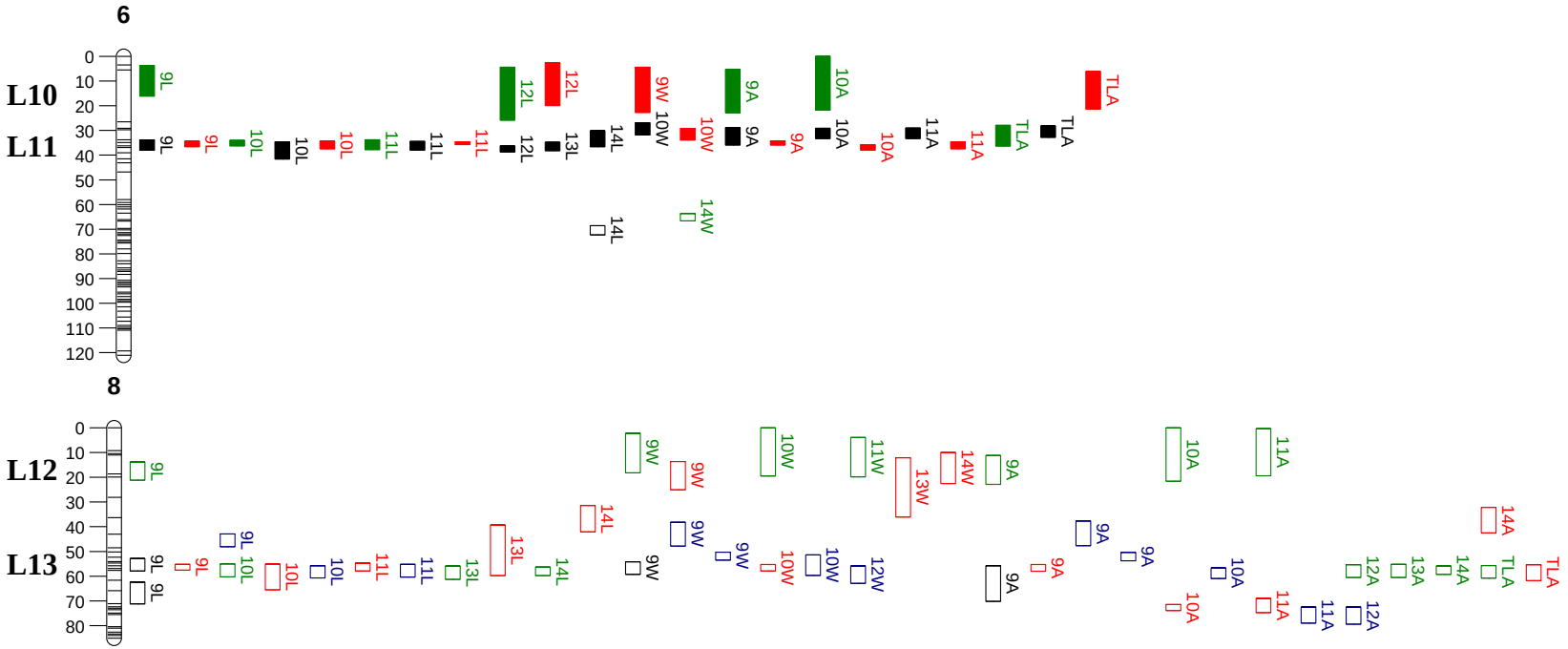


Figure 7.1, continued.

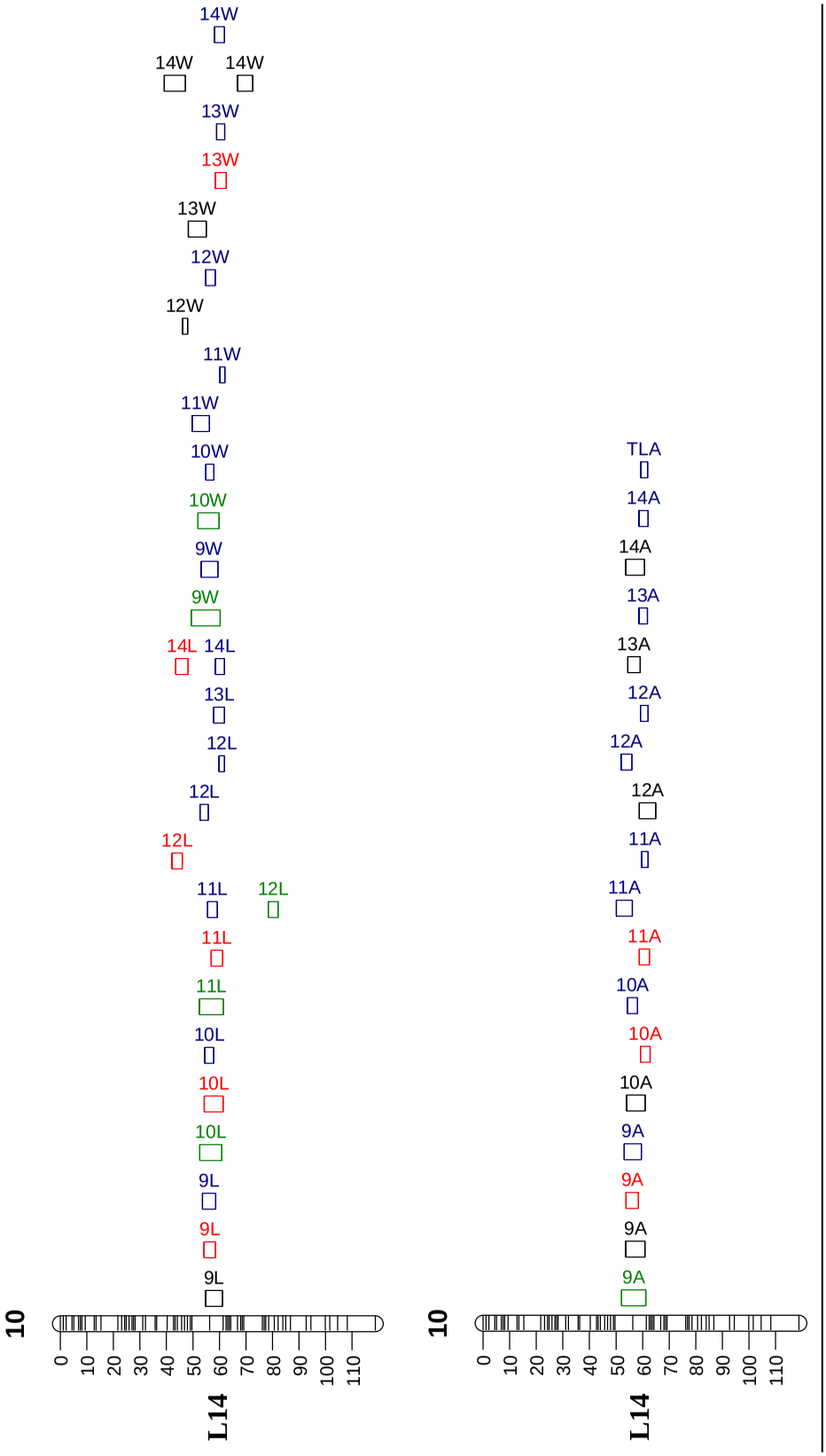


Figure 7.1, continued.

QTL that affect total leaf area were detected on all linkage groups except LG-07 and LG-09 (Fig. 7.1, Table 7.3). RTx7000 was responsible for increased leaf area associated with all of the QTL except in regions L3, 6, 13, and 14. All QTL for total leaf area co-localized with QTL for individual leaf size parameters. Region L6 total leaf area QTL co-localized with leaves 11-14 size parameters, regions L3, 5, 7, 9, and 10 aligned with leaves 9-12 size parameters, and regions L1, 4, 11, and 13 total leaf area QTL co-localized with size parameters of all leaves.

Table 7.3. Total leaf area QTL for BTx642 x RTx7000 RILs grown in field conditions in 2008, 2009, and 2010, and greenhouse conditions.

Env.	LG[†]	Region	Peak[‡]	LOD	LOD-1[§]	LOD-2[§]	a(H)[¶]	$R^{2\#}$	95% LOD
GH	1	L1	4.0	3.78	0.0 - 5.8	0.0 - 8.1	61.8	0.06	3.15
GH	2	L3	8.8	4.93	7.8 - 11.3	2.9 - 13.0	-70.1	0.07	3.15
2009	2	L4	116.6	3.61	111.4 - 120.0	106.0 - 120.9	111.7	0.09	2.98
2008	2	L4	123.8	4.17	117.4 - 126.0	111.0 - 126.0	143.2	0.13	3.03
2009	3	L5	24.1	4.06	22.4 - 28.1	22.3 - 38.2	119.4	0.10	2.98
2009	3	L5	40.5	3.36	38.1 - 43.7	22.2 - 44.2	112.3	0.09	2.98
GH	3	L6	114.2	3.16	113.0 - 116.6	102.4 - 118.5	-52.7	0.05	3.15
GH	4	L7	50.8	3.21	49.1 - 55.5	45.2 - 57.0	48.8	0.04	3.15
2008	5	L9	99.6	4.26	93.2 - 102.3	92.1 - 103.3	136.5	0.13	3.03
2009	5	L9	97.7	5.57	95.2 - 101.7	92.5 - 104.3	142.0	0.15	2.98
2010	5	L9	100.8	4.40	97.7 - 103.7	90.9 - 105.1	164.6	0.12	3.01
2010	6	L10	12.6	5.15	6.2 - 21.3	3.7 - 25.0	213.6	0.18	3.01
2008	6	L11	31.1	5.32	27.9 - 36.3	26.4 - 39.4	151.2	0.13	3.03
2009	6	L11	29.7	5.78	28.2 - 32.8	26.4 - 33.9	144.6	0.17	2.98
2010	8	L13	56.9	5.09	55.4 - 61.8	55.0 - 65.3	-183.7	0.13	3.01
2008	8	L13	56.9	4.07	55.7 - 60.8	54.9 - 65.6	-135.5	0.12	3.03
GH	10	L14	61.4	14.52	59.3 - 61.9	58.3 - 62.4	-176.6	0.31	3.15

[†]Linkage group.

[‡]QTL peak in cM.

[§]QTL width in cM at either 1 (LOD-1) or 2 (LOD-2) LOD units below the peak.

[¶]Additive effect (cm^2). Sign is with respect to RTx7000.

[#]Proportion of total phenotypic variance explained by the QTL.

Discussion

Analysis of the growth and development of leaves in BTx642 and RTx7000 in Chapter II revealed differences in leaf size that might contribute to variation in biomass accumulation, grain yield, or drought tolerance. Therefore, one overall goal of this study was to identify and characterize QTL for leaf length, width, and area. A second objective of this analysis was to determine the extent of correspondence between QTL for leaf traits identified through analysis of plants grown in the greenhouse and in the field. To accomplish these objectives, leaf length, width, and area were analyzed in the BTx642/RTx7000 RIL population grown in greenhouse pots and field conditions in 2008, 2009, and 2010.

QTL that modulate leaf size in the BTx642/RTx7000 RIL population were mapped to 14 major regions of the sorghum genome. BTx642 alleles in six QTL and RTx7000 alleles in eight QTL contributed to increased leaf size. QTL in several regions of the genome showed partially overlapping intervals and peak values in different environments, suggesting that these regions probably contain allelic variation in more than one gene that modulates the leaf trait analyzed. Therefore, 14 QTL is a minimum estimate of the number of QTL that modulate leaf size in this population. Lack of comprehensive detection of QTL was expected because only 90 RILs were analyzed and it is estimated that ~400 RILs are required to identify the majority of QTL in populations derived from a given cross (Beavis, 1998).

Analysis of the RIL population in greenhouse and field conditions enabled the detection of some QTL that are preferentially expressed in the different environments.

For example, leaf size QTL on LG-5 were observed only under field conditions whereas QTL in region L6 of LG-03 were observed preferentially in greenhouse pots under short days. Overall, this study identified QTL with large effects and larger population studies will be required for more comprehensive analysis of leaf size QTL.

Broad sense heritability of leaves 9-10 size traits was above 0.75 with respect to plants grown in field conditions and then declined by ~10-25% for leaf length, width, and area of leaves 13-14. Leaves 13 and 14 are the last leaves formed prior to anthesis. The growth of these leaves is affected by intrinsic factors, competition for resources by the rapidly developing peduncle and panicle, and environmental factors. Therefore, this may be one explanation for the lower relative heritability observed between leaves 13-14 and the lower leaves. Broad sense heritability was also markedly lower when analyzed across greenhouse and field conditions relative to across only field conditions. This is likely explained by the increased environmental variation observed across locations.

Leaf QTL and Flowering Time

Leaf size QTL mapped to six regions of the genome that overlap with the major QTL for flowering time (L1 and L2 on LG-01, L5 on LG-03, L11 on LG-06, L13 on LG-08, and L14 on LG-10; see Chapter V for flowering time loci). These QTL affect leaf traits associated with leaves nine through 14 that develop after plants complete the juvenile phase and during the phase of development when flowering time is modified by photoperiod and other factors. The study of BTx642 and RTx7000 leaf development in Chapter II showed that leaf size (length, width, and area) and weight increases with leaf number during most of plant development until the last few leaves produced prior to

anthesis. It is possible that the decrease in leaf size in the last few leaves produced prior to anthesis is due to the influence of the developing peduncle and panicle. A delay in flowering time results in a longer vegetative phase and the production of more and larger leaves prior to anthesis. All six QTL that modify flowering time overlap with leaf length, width, and area QTL for leaves nine through 14. The alleles that delay flowering time in the BTx642/RTx7000 RIL population are the same as those that increase leaf size. BTx642 alleles delay flowering and increase the size of leaves nine through 14 in regions L2, L13, and L14, whereas RTx7000 alleles delay flowering and increase leaf size in regions L1, L5, and l1. Therefore, following the juvenile phase, alleles that reduce time to flowering decrease canopy size (total leaf area) by decreasing the number of leaves produced and the relative size of the last several leaves expanded prior to anthesis. The combined impact of flowering time on these two leaf development parameters is consistent with the observed differential increase in total leaf area in BTx642 vs. RTx7000 between 77-87 DAE (Chapter II, Fig. 2.8) that is correlated with delayed flowering in BTx642 relative to RTx7000. While delayed flowering time is highly correlated with increased upper leaf size, it is possible that QTL for leaf size are located in the same interval as flowering time QTL. Therefore, higher resolution QTL maps and analysis of the genes and alleles that underlie the QTL identified here will be required to directly test this association between flowering time and leaf size.

QTL That Modify Leaf Size and Dimensions

QTL for leaf length, width and area were co-located in eight regions of the sorghum genome in addition to the six regions affected by flowering time (Fig. 7.1, L3,

4, 6, 7-9, 10, and 12). RTx7000 alleles in five of these loci increased leaf length, width, and area of leaves eight through 14, whereas BTx642 alleles in the three remaining loci increased these traits. These QTL enable modulation of overall leaf size during development irrespective of flowering time. Combinations of these alleles could result in leaves with a wide range of dimensions and area. As shown in Table 7.4 RTx7000 had greater leaf length and area than BTx642 with respect to upper canopy leaves 10-12 and greater leaf width in leaves 11-12 under field conditions in 2010. The RILs differed by 1.5-3.5 fold across the population for leaf size parameters. RILs with transgressive segregation for leaf length, width and area were observed in the population. Table 7.5 shows the genotypes spanning leaf size QTL for the three RILs with the largest upper canopy. RILs containing combinations of BTx642 and RTx7000 alleles for leaf size QTL produced the largest leaves.

Table 7.4. Upper leaf size parameters for BTx642 x RTx7000 parental and recombinant inbred lines (RILs) grown in field conditions in 2010.

Leaf	BTx642	RTx7000	RILs
	Mean	Mean	Range
Length (cm)			
10	74.0	74.6	48.8-90.3
11	73.7	83.0	53.2-88.9
12	72.4	83.7	59.6-88.2
Width (cm)			
10	10.2	9.1	6.1-12.6
11	9.5	10.2	7.4-12.9
12	10.4	10.6	7.7-12.3
Area (cm^2)			
10	392.0	397.6	176.3-615.5
11	399.1	465.1	237.7-619.7
12	390.7	478.6	282.8-576.3

Table 7.5. Genotypic profile of leaf size parameter loci for leaves 10-12 of BTx642 x RTx7000 RILs with the largest leaves.[†]

Region	RIL		
	33	60	42
L2			
L3			
L4			
L7			
L8			
L9			
L10			
L11			
L13			
L14			

[†]Green shading indicates BTx642, yellow RTx7000, and mixed indicates both genotypes in the region.

CHAPTER VIII

GENETIC BASIS OF ROOT TRAIT VARIATION IN BTx642 AND RTx7000

Introduction

The root functions in the plant as the primary source of water and mineral acquisition and depending on the species, as a primary or secondary organ for assimilate storage. These functions are achieved through five distinct structures that make up the root: the epidermis, cortex, endodermis, pericycle, and stele. The epidermis forms the outer boundary of the root and serves as both a protective layer for underlying tissues and as a barrier to water loss in mature areas of the root system. In actively growing regions of the root system, the epidermis functions in water and mineral uptake. In pumpkin for example, Kramer and Boyer (1995) found this region to span the last 80 mm of the actively growing root. The epidermis covers the root cortex, which is composed of loosely packed cells that function both in water movement from the epidermis to vascular tissues and as a site of assimilate storage. Bordering the internal side of the cortex is the endodermis which forms a boundary between the cortex and pericycle. Cells of the endodermis are lined with the Casparian strip, a layer of suberin that is highly hydrophobic and forces water and solute movement between the cortex and stele to pass through endodermal cells instead of around them. On the interior side of the endodermis is the pericycle, which functions as a secondary meristem for the root through which lateral roots are formed. At the very interior of the root is the stele. The stele is made up of vascular tissues xylem and phloem. The xylem facilitates water and mineral movement through the root while the phloem facilitates photosynthate

movement. Vascular tissues in the roots are connected to the vascular system of the stem and leaves, allowing for movement of water, minerals, and photosynthate between areas of active growth, photosynthate production, and storage.

Water movement through the root can occur through three known pathways: the apoplast, transmembrane, and symplast pathways (Frensch et al., 1996; Steudle and Frensch, 1996; Canny, 1998). In apoplastic movement water moves through the cell walls of cortex cells, bypassing all cellular membranes. In transmembrane movement, water passes entirely through the cell, through both sides of the plasma membrane. In both apoplastic and transmembrane pathways, water movement ceases at the endodermis where passage is blocked by the Casparian strip. At this point, further water movement to the stele is restricted to passage through the symplast. The symplast is composed of a network of channels called plasmodesmata that form connections between cells from one plasma membrane to the next, allowing water and mineral passage. Movement through this pathway can occur through all tissues of the root.

Cereal roots are classified as fibrous as opposed to the taproot system of legumes. Their morphology is comprised of several components including seminal, nodal, and lateral roots (Fageria et al., 1997). The seminal or primary root comes from primordia in the seed and is the first root of the plant. Lateral, or secondary roots arise from the primary root. Continued branching of secondary roots leads to tertiary, quaternary, etc. roots (Tatsumi et al., 1989). Cereals are also able to develop roots from stem meristems that are known as nodal or crown roots. Nodal roots that penetrate the soil and produce lateral roots have been classified as functional roots since they aid the

plant in water and nutrient absorption, whereas nodal roots that serve only to support shoot biomass have been classified as non-functional or brace roots (Thomas and Kaspar, 1997).

Root morphology can be affected by numerous factors including soil composition, compaction, nutrient and moisture content, pH, weather factors such as temperature and wind, biological factors such as microbes, insects, and other plants, and genotype diversity (Fitter, 1982). Nutrient and moisture stress modulation of root growth and function are particularly important. Nutrient-deficient plants, especially plants lacking sufficient amounts of nitrogen, have greater root-shoot weight ratios than controls, indicating that the plant is increasing root growth as a mechanism to increase nitrogen uptake. In wheat, Talouizte et al. (1984) showed that wheat seedlings at 18 DAE had much higher concentrations of soluble carbohydrates in their root systems than control plants. In maize, in addition to having higher root-shoot weight ratios early in development under nitrogen deprivation (Eghball et al., 1993), high root sink strength at anthesis resulted in plants with large root systems but low grain yield (Barber, 1995). Drought stress has been shown to have similar effects on the root system, with increased root-shoot weight ratios under drought stress. Mackay and Barber (1985) showed that in maize at 28 DAE subjected to well-watered conditions at 0.32 m^3 water/m^3 soil and then progressively dried to 0.22 m^3 water/m^3 soil, root-shoot weight ratios increased from 0.18 to 0.27. Studies in other cereals have found similar results (Gregory et al., 1995; Khan et al., 2002; Dhanda et al., 2004).

In this chapter variation in root traits including root length, surface area, and volume were examined in the BTx642 x RTx7000 RIL population under well-watered conditions in the field at 52 DAE in 2009 and 2010. QTL analysis identified several genomic loci that modulate expression of these root traits.

Results

Root size traits were examined among the BTx642 x RTx7000 RIL population and parental lines BTx642 and RTx7000 grown under field conditions in 2009 and 2010 to 52 DAE. Root systems were excavated from the field and washed thoroughly to remove soil and organic matter from the roots prior to scanning with WinRhizo V.2008a software (Regent Instruments). This program analyzed a given root system by first measuring the diameter of each individual root across its length and then dividing roots into classes based on root diameter. Nine root classes were formed, starting with class one describing roots 0.0-0.5 mm in diameter. Class numbers incrementally increased by 0.5 mm in root diameter up until class nine, which described roots 4.5 mm and greater in diameter. Within a given class, roots were further classified with respect to root length, surface area, volume, and the number of root tips. On a global scale, root length, surface area, volume, and the number of root tips were summed across all classes. Additionally, the extent of root branching was measured for a given root system by counting the number of forks (junctions where new lateral roots arise). Root fresh and dry weights were also measured in this experiment.

Table 8.1 shows that RTx7000 in 2009 produced a root system with greater total root surface area (277.8 cm^2 vs. 221.5 cm^2) and total root volume (14.33 cm^3 vs. 10.51 cm^3) relative to BTx642. The larger RTx7000 root system can be attributed to more root tissue in the 1.5-3.5 mm diameter range (classes four through seven). In 2010 these differences were diminished, with both parents exhibiting lower trait values for most traits. Other root traits were similar in RTx7000 and BTx642 in both years at this stage of development in the field including the total number of root tips and forks, and root weight. With respect to the RILs, means for the population were either within or slightly above parental means for most traits. For a few traits, such as class one root size parameters, class one root tip number, and the total number of forks, RIL population means were well above parental means – more so in 2009 than 2010. Variation across the population ranged from 1.5-11.0 fold in 2009 and 2.2-8.0 fold in 2010, with root tip number classes exhibiting the largest variation.

Across years in the field, rep, genotype, and environment were significant sources of variation for most traits (Table 8.2). Genotype accounted for on average ~10-20% of total variation, while residual variation was on average ~40-80% across traits. Broad sense heritability was highest for root fresh weight at 0.52. For most traits, broad sense heritability ranged from ~0.30-0.40. Root size parameters (length, surface area, and volume) for the largest diameter classes of roots showed the highest heritability.

Table 8.1. Root size parameters for BTx642 x RTx7000 parental and recombinant inbred lines (RILs) grown in the field in 2009 and 2010.

Trait	Year	BTx642	RTx7000	RIL Population		Trait	Year	BTx642	RTx7000	RIL Population	
		Mean	Mean	Mean (SD†)	Min.-Max.			Mean	Mean	Mean (SD†)	Min.-Max.
Root Weight (g)											
Fresh	2009	58.52	52.90	47.90 (10.82)	25.11-85.67	Dry	2009	9.93	9.90	8.57 (2.12)	3.90-15.86
	2010	33.75	30.07	44.58 (10.75)	22.49-86.12		2010	5.26	4.13	6.65 (1.70)	3.40-13.48
Root Length (cm)											
1	2009	565.9	568.5	737.7 (195.5)	355.3-1140.9	6	2009	27.2	41.2	35.8 (10.1)	14.3-63.5
	2010	417.8	365.8	456.3 (107.7)	255.0-834.3		2010	31.6	21.7	26.0 (6.9)	13.0-53.4
2	2009	192.5	197.6	191.9 (36.3)	120.8-284.5	7	2009	20.2	31.3	25.2 (7.9)	7.0-46.1
	2010	136.3	129.7	144.5 (25.3)	102.5-229.7		2010	20.5	17.6	20.3 (5.5)	10.4-45.6
3	2009	66.2	63.9	72.4 (14.1)	36.4-103.5	8	2009	16.7	20.4	17.2 (5.7)	5.9-32.4
	2010	64.7	58.7	72.9 (13.7)	49.5-110.7		2010	12.7	13.2	14.6 (3.9)	6.5-31.4
4	2009	40.7	58.9	52.1 (9.8)	32.6-74.7	9	2009	11.3	13.7	12.4 (4.4)	3.5-25.0
	2010	46.7	40.8	48.1 (10.8)	28.1-75.4		2010	9.0	8.3	11.5 (3.0)	6.7-23.5
5	2009	29.0	53.6	45.3 (11.7)	23.6-73.1	Total	2009	969.4	1049.1	1190.1 (277.9)	613.3-1734.1
	2010	38.5	31.2	39.5 (9.2)	20.7-63.5		2010	777.9	686.8	833.5 (163.8)	524.3-1354.0
Root SA (cm^2)											
1	2009	34.7	33.5	41.6 (10.5)	21.3-63.1	6	2009	23.3	35.4	30.7 (8.7)	12.4-54.6
	2010	24.4	20.4	26.4 (6.1)	14.5-48.8		2010	27.3	18.7	22.4 (5.9)	11.2-46.1
2	2009	41.2	41.2	40.9 (7.7)	26.1-60.1	7	2009	20.5	31.7	25.7 (8.1)	7.2-46.9
	2010	30.9	29.3	32.7 (5.7)	22.9-52.5		2010	20.7	17.8	20.5 (5.6)	10.5-46.1
3	2009	25.1	24.2	27.5 (5.3)	13.9-39.4	8	2009	19.6	24.0	20.2 (6.8)	6.9-38.0
	2010	24.7	22.5	27.9 (5.3)	19.1-42.1		2010	14.9	15.5	17.1 (4.5)	7.7-36.9
4	2009	21.9	32.3	28.3 (5.4)	17.7-40.9	9	2009	15.0	18.3	16.6 (5.9)	4.7-33.4
	2010	25.7	22.4	26.4 (5.9)	15.3-41.5		2010	12.0	11.0	15.3 (4.0)	8.9-31.3
5	2009	20.2	37.3	31.8 (8.3)	16.5-51.6	Total	2009	221.5	277.8	263.2 (58.6)	134.5-398.5
	2010	27.2	22.0	27.9 (6.5)	14.6-44.7		2010	207.8	179.6	216.6 (42.0)	142.9-355.9
Root Volume (cm^3)											
1	2009	0.24	0.23	0.27 (0.07)	0.14-0.41	6	2009	1.60	2.42	2.11 (0.59)	0.85-3.75
	2010	0.16	0.13	0.18 (0.04)	0.10-0.33		2010	1.88	1.28	1.54 (0.40)	0.77-3.17
2	2009	0.73	0.71	0.72 (0.14)	0.47-1.05	7	2009	1.66	2.56	2.08 (0.65)	0.58-3.81
	2010	0.58	0.55	0.61 (0.11)	0.42-0.99		2010	1.66	1.44	1.65 (0.45)	0.85-3.71
3	2009	0.77	0.74	0.84 (0.16)	0.43-1.21	8	2009	1.84	2.24	1.90 (0.63)	0.64-3.56
	2010	0.76	0.70	0.86 (0.16)	0.59-1.29		2010	1.39	1.46	1.60 (0.42)	0.72-3.45
4	2009	0.94	1.42	1.23 (0.23)	0.77-1.80	9	2009	1.60	1.94	1.77 (0.63)	0.50-3.56
	2010	1.13	0.98	1.16 (0.26)	0.67-1.83		2010	1.27	1.17	1.63 (0.42)	0.94-3.52
5	2009	1.13	2.08	1.78 (0.47)	0.92-2.91	Total	2009	10.51	14.33	12.69 (3.24)	5.94-21.42
	2010	1.54	1.24	1.58 (0.37)	0.83-2.52		2010	10.37	8.95	10.81 (2.37)	6.32-19.88
Root Tips											
1	2009	4789	4178	6515 (2196)	2562-12286	6	2009	3.3	3.0	3.2 (1.3)	1-9
	2010	3381	4146	4332 (1152)	2251-10144		2010	0.8	1.5	1.7 (0.7)	0-4
2	2009	74.0	61.0	75.2 (18.4)	37-121	7	2009	1.7	3.0	2.3 (1.0)	1-6
	2010	49.8	46.0	53.8 (10.8)	34-105		2010	1.5	1.0	1.4 (0.7)	0-4
3	2009	16.7	19.5	16.1 (4.4)	8-28	8	2009	1.0	1.5	1.6 (0.9)	0-4
	2010	15.8	11.7	12.5 (2.9)	8-27		2010	0.8	1.0	0.8 (0.4)	0-2
4	2009	6.3	6.5	7.2 (2.2)	3-13	9	2009	2.0	2.0	1.0 (0.6)	0-3
	2010	3.8	4.8	5.1 (1.4)	3-12		2010	0.2	0.7	0.6 (0.4)	0-2
5	2009	2.7	4.5	4.5 (1.8)	1-11	Total	2009	4896	4279	6626 (2222)	2616-12464
	2010	1.7	3.5	3.0 (1.1)	0-8		2010	3455	4216	4411 (1167)	2309-10308
Root Forks	2009	8180.0	7502.0	10554 (3949)	3585-19224						
	2010	4868.0	4593.0	5785 (1819)	2778-16182						

†Standard deviation.

Table 8.2. Analysis of variance across field conditions in 2009-2010 for root size parameters in the BTx642 x RTx7000 RIL population.

Trait[†]	Source	df	MS[‡]		SS[§]	CoV[¶]	TV%[#]
RFW	Rep	1	1.01E-01		1.63E-06	6.10E-01	0.3
	G	89	3.13E+02	***	4.51E-01	4.05E+01	22.9
	E	1	9.86E+02	**	1.59E-02	4.66E+00	2.6
	GxE	89	1.52E+02	*	2.19E-01	2.14E+01	12.1
H=0.52	Error	178	1.09E+02		3.15E-01	1.09E+02	62.0
RL1	Rep	1	1.99E+05	*	9.21E-03	9.41E+02	1.2
	G	89	5.98E+04	*	2.46E-01	5.23E+03	6.5
	E	1	7.03E+06	***	3.25E-01	3.93E+04	48.6
	GxE	89	3.90E+04		1.61E-01	3.76E+03	4.7
H=0.35	Error	177	3.16E+04		2.59E-01	3.16E+04	39.1
RL2	Rep	1	1.31E+04	***	1.74E-02	6.76E+01	2.5
	G	89	2.37E+03	**	2.80E-01	2.13E+02	7.9
	E	1	1.99E+05	***	2.64E-01	1.11E+03	41.0
	GxE	89	1.53E+03	*	1.80E-01	2.13E+02	7.9
H=0.36	Error	177	1.10E+03		2.59E-01	1.10E+03	40.8
RL3	Rep	1	4.47E+03	***	3.67E-02	2.35E+01	6.6
	G	89	4.76E+02	**	3.48E-01	4.63E+01	13.0
	E	1	2.22E+01		1.82E-04	1.52E+00	0.4
	GxE	89	2.92E+02		2.14E-01	8.49E+00	2.4
H=0.39	Error	177	2.75E+02		4.01E-01	2.75E+02	77.5
RL4	Rep	1	5.16E+03	***	6.99E-02	2.80E+01	12.2
	G	89	2.57E+02	**	3.10E-01	2.33E+01	10.2
	E	1	1.38E+03	**	1.87E-02	6.81E+00	3.0
	GxE	89	1.64E+02		1.98E-01	2.27E+00	1.0
H=0.39	Error	177	1.68E+02		4.04E-01	1.68E+02	73.6
RL5	Rep	1	3.59E+03	***	4.88E-02	1.93E+01	8.6
	G	89	2.72E+02	*	3.29E-01	2.63E+01	11.8
	E	1	2.85E+03	***	3.87E-02	1.51E+01	6.7
	GxE	89	1.67E+02		2.02E-01	4.33E+00	1.9
H=0.39	Error	177	1.59E+02		3.82E-01	1.59E+02	70.9
RL6	Rep	1	2.14E+03	***	3.70E-02	1.14E+01	5.4
	G	89	1.90E+02	**	2.92E-01	2.18E+01	10.5
	E	1	8.33E+03	***	1.44E-01	4.62E+01	22.2
	GxE	89	1.04E+02		1.59E-01	8.54E+00	4.1
H=0.39	Error	177	1.21E+02		3.68E-01	1.21E+02	57.8
RL7	Rep	1	1.39E+03	***	4.25E-02	7.42E+00	6.9
	G	89	1.21E+02	**	3.30E-01	1.42E+01	13.2
	E	1	2.19E+03	***	6.68E-02	1.19E+01	11.1
	GxE	89	6.49E+01		1.77E-01	3.05E+00	2.8
H=0.43	Error	177	7.10E+01		3.84E-01	7.10E+01	66.0
RL8	Rep	1	4.14E+02	***	2.67E-02	2.14E+00	4.6
	G	89	6.10E+01	**	3.50E-01	6.63E+00	14.3
	E	1	6.02E+02	***	3.88E-02	3.19E+00	6.9
	GxE	89	3.47E+01		1.99E-01	4.66E-01	1.0
H=0.43	Error	177	3.38E+01		3.85E-01	3.38E+01	73.1
RL9	Rep	1	1.11E+02	*	1.26E-02	5.11E-01	2.0
	G	89	3.61E+01	**	3.64E-01	3.86E+00	15.3
	E	1	7.49E+01		8.48E-03	3.04E-01	1.2
	GxE	89	2.08E+01		2.10E-01	2.80E-01	1.1
H=0.43	Error	177	2.02E+01		4.06E-01	2.02E+01	80.3

Trait[†]	Source	df	MS[‡]		SS[§]	CoV[¶]	TV%[#]
RDW	Rep	1	8.98E-02		3.82E-05	2.16E-02	0.3
	G	89	9.06E+00	**	3.43E-01	8.39E-01	11.2
	E	1	3.30E+02	***	1.41E-01	1.81E+00	24.2
	GxE	89	5.72E+00	**	2.17E-01	8.88E-01	11.8
H=0.37	Error	178	3.95E+00		2.99E-01	3.95E+00	52.6
RSA1	Rep	1	9.15E+02	**	1.43E-02	4.62E+00	1.9
	G	89	1.74E+02	*	2.43E-01	1.41E+01	5.9
	E	1	2.04E+04	***	3.19E-01	1.14E+02	47.7
	GxE	89	1.18E+02		1.65E-01	1.27E+01	5.3
H=0.32	Error	177	9.32E+01		2.59E-01	9.32E+01	39.1
RSA2	Rep	1	6.00E+02	***	1.85E-02	3.07E+00	2.8
	G	89	1.12E+02	*	3.08E-01	1.04E+01	9.6
	E	1	5.98E+03	***	1.84E-01	3.32E+01	30.4
	GxE	89	7.08E+01		1.94E-01	8.50E+00	7.8
H=0.37	Error	177	5.39E+01		2.94E-01	5.39E+01	49.4
RSA3	Rep	1	7.23E+02	***	4.07E-02	3.84E+00	7.4
	G	89	6.89E+01	**	3.45E-01	6.61E+00	12.7
	E	1	1.94E+01		1.09E-03	1.31E-01	0.3
	GxE	89	4.26E+01		2.14E-01	1.32E+00	2.5
H=0.38	Error	177	4.00E+01		3.99E-01	4.00E+01	77.1
RSA4	Rep	1	1.56E+03	***	7.06E-02	8.48E+00	12.4
	G	89	7.73E+01	**	3.11E-01	7.03E+00	10.3
	E	1	2.93E+02	**	1.33E-02	1.37E+00	2.0
	GxE	89	4.94E+01		1.99E-01	6.34E-01	0.9
H=0.35	Error	177	5.07E+01		4.06E-01	5.07E+01	74.3
RSA5	Rep	1	1.79E+03	***	4.89E-02	9.60E+00	8.7
	G	89	1.36E+02	**	3.31E-01	1.32E+01	11.9
	E	1	1.28E+03	***	3.50E-02	6.72E+00	6.1
	GxE	89	8.33E+01		2.03E-01	2.21E+00	2.0
H=0.39	Error	177	7.89E+01		3.82E-01	7.89E+01	71.3
RSA6	Rep	1	1.59E+03	***	3.71E-02	8.43E+00	5.5
	G	89	1.41E+02	**	2.92E-01	1.61E+01	10.5
	E	1	6.10E+03	***	1.42E-01	3.38E+01	22.0
	GxE	89	7.68E+01		1.59E-01	6.31E+00	4.1
H=0.39	Error	177	8.93E+01		3.69E-01	8.93E+01	58.0
RSA7	Rep	1	1.43E+03	***	4.24E-02	7.62E+00	6.9
	G	89	1.25E+02	**	3.30E-01	1.46E+01	13.2
	E	1	2.32E+03	***	6.89E-02	1.27E+01	11.4
	GxE	89	6.69E+01		1.76E-01	3.05E+00	2.7
H=0.43	Error	177	7.29E+01		3.83E-01	7.29E+01	65.8
RSA8	Rep	1	5.66E+02	***	2.64E-02	2.92E+00	4.6
	G	89	8.44E+01	**	3.50E-01	9.20E+00	14.4
	E	1	8.59E+02	***	4.00E-02	4.55E+00	7.1
	GxE	89	4.78E+01		1.98E-01	5.13E-01	0.8
H=0.44	Error	177	4.68E+01		3.86E-01	4.68E+01	73.1
RSA9	Rep	1	1.97E+02	*	1.25E-02	9.01E-01	2.0
	G	89	6.44E+01	**	3.64E-01	6.91E+00	15.4
	E	1	1.39E+02		8.81E-03	5.73E-01	1.3
	GxE	89	3.70E+01		2.09E-01	4.04E-01	0.9
H=0.43	Error	177	3.62E+01		4.06E-01	3.62E+01	80.5

Table 8.2, continued.

Trait[†]	Source	df	MS[‡]		SS[§]	CoV[¶]	TV%[#]
TRL	Rep	1	7.64E+05	***	1.82E-02	3.92E+03	2.6
	G	89	1.27E+05	**	2.68E-01	1.17E+04	7.7
	E	1	1.12E+07	***	2.68E-01	6.27E+04	41.5
H=0.37	GxE	89	8.02E+04		1.70E-01	7.32E+03	4.8
	Error	177	6.57E+04		2.76E-01	6.57E+04	43.4
RV1	Rep	1	4.40E-02	***	1.72E-02	2.25E-04	2.4
	G	89	7.05E-03	*	2.45E-01	5.54E-04	5.9
	E	1	7.57E-01	***	2.96E-01	4.22E-03	44.9
H=0.31	GxE	89	4.86E-03		1.69E-01	4.62E-04	4.9
	Error	177	3.94E-03		2.73E-01	3.94E-03	41.9
RV2	Rep	1	1.86E-01	**	1.89E-02	9.43E-04	3.0
	G	89	3.70E-02	*	3.35E-01	3.58E-03	11.5
	E	1	1.08E+00	***	1.10E-01	5.93E-03	19.1
H=0.39	GxE	89	2.28E-02		2.06E-01	2.24E-03	7.2
	Error	177	1.83E-02		3.30E-01	1.83E-02	59.1
RV3	Rep	1	7.58E-01	***	4.47E-02	4.05E-03	8.2
	G	89	6.52E-02	**	3.42E-01	6.21E-03	12.5
	E	1	4.93E-02		2.90E-03	4.90E-05	0.1
H=0.38	GxE	89	4.05E-02		2.13E-01	1.25E-03	2.5
	Error	177	3.81E-02		3.97E-01	3.81E-02	76.7
RV4	Rep	1	3.02E+00	***	7.10E-02	1.64E-02	12.5
	G	89	1.49E-01	*	3.12E-01	1.36E-02	10.4
	E	1	3.84E-01	*	9.02E-03	1.62E-03	1.2
H=0.35	GxE	89	9.56E-02		2.00E-01	1.23E-03	0.9
	Error	177	9.80E-02		4.08E-01	9.80E-02	74.9
RV5	Rep	1	5.69E+00	***	4.91E-02	3.06E-02	8.7
	G	89	4.34E-01	*	3.33E-01	4.25E-02	12.1
	E	1	3.67E+00	***	3.16E-02	1.91E-02	5.5
H=0.39	GxE	89	2.65E-01		2.03E-01	7.11E-03	2.0
	Error	177	2.51E-01		3.83E-01	2.51E-01	71.6
RV6	Rep	1	7.57E+00	***	3.75E-02	4.02E-02	5.6
	G	89	6.63E-01	**	2.92E-01	7.54E-02	10.4
	E	1	2.84E+01	***	1.41E-01	1.58E-01	21.8
H=0.39	GxE	89	3.63E-01		1.60E-01	2.91E-02	4.0
	Error	177	4.21E-01		3.69E-01	4.21E-01	58.2
RV7	Rep	1	9.28E+00	***	4.20E-02	4.95E-02	6.8
	G	89	8.17E-01	**	3.29E-01	9.57E-02	13.2
	E	1	1.57E+01	***	7.11E-02	8.58E-02	11.8
H=0.43	GxE	89	4.38E-01		1.76E-01	1.96E-02	2.7
	Error	177	4.77E-01		3.82E-01	4.77E-01	65.5
RV8	Rep	1	4.94E+00	***	2.61E-02	2.54E-02	4.5
	G	89	7.41E-01	**	3.49E-01	8.09E-02	14.4
	E	1	7.79E+00	***	4.12E-02	4.14E-02	7.3
H=0.44	GxE	89	4.20E-01		1.98E-01	4.23E-03	0.8
	Error	177	4.11E-01		3.86E-01	4.12E-01	73.0
RV9	Rep	1	2.19E+00	*	1.23E-02	1.00E-02	2.0
	G	89	7.30E-01	**	3.63E-01	7.81E-02	15.3
	E	1	1.64E+00		9.16E-03	6.84E-03	1.3
H=0.43	GxE	89	4.20E-01		2.09E-01	4.72E-03	0.9
	Error	177	4.10E-01		4.06E-01	4.10E-01	80.5
TRSA	Rep	1	7.78E+04	***	4.30E-02	4.18E+02	7.2
	G	89	6.48E+03	***	3.19E-01	6.59E+02	11.3
	E	1	1.90E+05	***	1.05E-01	1.05E+03	18.0
H=0.41	GxE	89	3.87E+03		1.90E-01	1.78E+02	3.1
	Error	177	3.51E+03		3.43E-01	3.51E+03	60.4
Tips1	Rep	1	2.37E+07	*	9.89E-03	1.05E+05	1.3
	G	89	7.29E+06	*	2.71E-01	6.07E+05	7.6
	E	1	4.22E+08	***	1.76E-01	2.34E+06	29.5
H=0.33	GxE	89	4.88E+06		1.81E-01	4.15E+03	0.1
	Error	177	4.89E+06		3.61E-01	4.89E+06	61.5
Tips2	Rep	1	1.79E+03	**	1.03E-02	8.40E+00	1.4
	G	89	5.54E+02	**	2.82E-01	5.29E+01	8.7
	E	1	4.05E+04	***	2.31E-01	2.25E+02	37.1
H=0.38	GxE	89	3.44E+02		1.75E-01	2.31E+01	3.8
	Error	177	2.98E+02		3.01E-01	2.98E+02	49.0
TIPS3	Rep	1	1.92E+01		2.02E-03	1.36E-03	<0.1
	G	89	3.16E+01		2.95E-01	2.07E+00	6.9
	E	1	1.17E+03	***	1.23E-01	6.44E+00	21.5
H=0.26	GxE	89	2.34E+01		2.19E-01	1.98E+00	6.6
	Error	177	1.95E+01		3.62E-01	1.95E+01	65.0
Tips4	Rep	1	5.56E+00		2.15E-03	8.23E-04	<0.1
	G	89	7.60E+00		2.61E-01	2.72E-01	3.3
	E	1	3.72E+02	***	1.43E-01	2.05E+00	24.7
H=0.14	GxE	89	6.52E+00		2.24E-01	5.54E-01	6.7
	Error	177	5.42E+00		3.70E-01	5.42E+00	65.3
Tips5	Rep	1	6.39E+00		4.06E-03	1.76E-02	0.4
	G	89	5.44E+00	**	3.07E-01	5.05E-01	10.1
	E	1	2.02E+02	***	1.28E-01	1.12E+00	22.4
H=0.37	GxE	89	3.44E+00		1.94E-01	8.74E-02	1.8
	Error	177	3.26E+00		3.66E-01	3.26E+00	65.4
Tips6	Rep	1	6.66E+00		7.31E-03	2.74E-02	0.9
	G	89	2.28E+00		2.23E-01	2.34E-02	0.8
	E	1	1.91E+02	***	2.10E-01	1.06E+00	34.3
H=0.41	GxE	89	2.19E+00		2.14E-01	2.04E-01	6.6
	Error	177	1.78E+00		3.46E-01	1.78E+00	57.5
Tips7	Rep	1	3.74E+00		7.03E-03	1.42E-02	0.8
	G	89	1.46E+00		2.43E-01	4.34E-02	2.6
	E	1	6.93E+01	***	1.30E-01	3.82E-01	22.6
H=0.12	GxE	89	1.28E+00		2.15E-01	3.33E-02	2.0
	Error	177	1.22E+00		4.05E-01	1.22E+00	72.0
Tips8	Rep	1	1.36E+00		3.55E-03	2.69E-03	0.2
	G	89	1.14E+00	*	2.65E-01	9.25E-02	6.9
	E	1	5.52E+01	***	1.44E-01	3.06E-01	22.9
H=0.27	GxE	89	7.74E-01		1.80E-01	5.35E-02	4.0
	Error	177	8.80E-01		4.07E-01	8.80E-01	66.0
Tips9	Rep	1	7.60E-02		4.11E-04	2.08E-03	0.3
	G	89	6.29E-01	*	3.03E-01	5.60E-02	9.4
	E	1	1.35E+01	***	7.31E-02	7.36E-02	12.3
H=0.32	GxE	89	4.07E-01		1.96E-01	1.98E-02	3.3
	Error	177	4.46E-01		4.27E-01	4.46E-01	74.7

Table 8.2, continued.

Trait[†]	Source	df	MS[‡]		SS[§]	CoV[¶]	TV%[#]
TRV	Rep	1	2.29E+02	***	4.24E-02	1.22E+00	7.4
	G	89	2.06E+01	**	3.39E-01	2.28E+00	13.7
	E	1	3.09E+02	***	5.72E-02	1.67E+00	10.1
	GxE	89	1.15E+01		1.90E-01	8.97E-02	0.5
H=0.44	Error	177	1.13E+01		3.72E-01	1.13E+01	68.3
Forks	Rep	1	7.84E+07	*	1.01E-02	3.67E+05	1.3
	G	89	2.21E+07		2.53E-01	1.62E+06	5.9
	E	1	2.01E+09	***	2.60E-01	1.12E+07	40.7
	GxE	89	1.56E+07		1.79E-01	1.28E+06	4.6
H=0.29	Error	177	1.31E+07		2.98E-01	1.31E+07	47.4

Trait[†]	Source	df	MS[‡]		SS[§]	CoV[¶]	TV%[#]
TTips	Rep	1	2.42E+07	*	9.89E-03	1.08E+05	1.3
	G	89	7.46E+06	*	2.71E-01	6.21E+05	7.6
	E	1	4.34E+08	***	1.77E-01	2.41E+06	29.6
	GxE	89	5.00E+06		1.81E-01	3.35E+03	0.0
H=0.33	Error	177	4.99E+06		3.60E-01	4.99E+06	61.3

***Indicates significance at the 0.001, **0.01, and *0.05 probability levels.

[†]Root fresh weight (RFW), root dry weight (RDW), root length (RL), root surface area (RSA), root volume (RV), number of root tips (Tips), number of root forks (Forks), broad sense heritiability (*H*). A "T" before a trait name denotes the total of all classes. A numeral following a trait denotes the diameter of the root constituting the class in increments of 0.5 mm, starting at 0.5 mm.

[‡]Mean square.

[§]Proportion of total sums of squares.

[¶]Covariate value.

[#]Percentage of total variation.

QTL Analysis

QTL analysis was conducted on root traits using data collected from RILs grown to 52 DAE under field conditions in 2009 and 2010. QTL were detected in 16 regions of the genome, spanning all linkage groups except LG-09 (Fig. 8.1, Appendix B). QTL were given designations based on the region of the genome they were associated with (R1-R16). Only major regions were labeled; however, single QTL not clustering with others are still shown on the map. LOD thresholds at the 95% confidence level for root size parameter QTL ranged from 2.64 to 3.32 (Appendix B). Individual QTL on average explained between 8% and 23% of the phenotypic variance. QTL significance at the peak ranged from LOD 3.09 to 6.31.

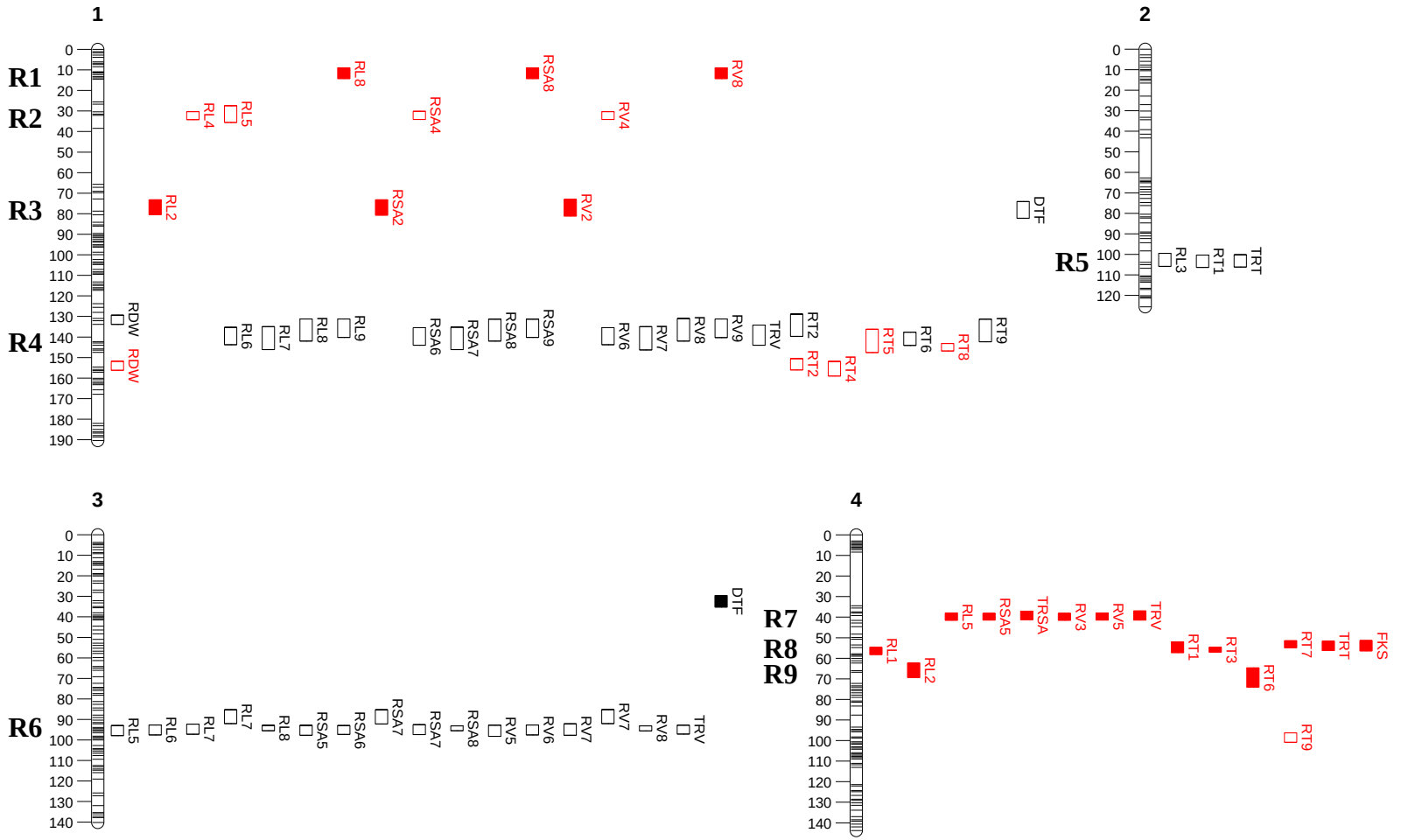


Figure 8.1. Quantitative trait loci positions for root size parameter and flowering time in the BTx642 x RTx7000 RIL population. QTL are shown for root length (RL), root surface area (RSA), root volume (RV), number of root tips (RT), number of root forks (FKS), root fresh (RFW) or dry weight (RDW), and flowering time (DTF). Roots were divided into classes based on root diameter, starting at 1, (0.0-0.5 mm) and stepping incrementally by 0.5 mm to class 9 (≥4.5 mm). Traits with a "T" denote summation of classes for the respective trait. Filled bars denote QTL where RTx7000 increased the magnitude of the trait; unfilled bars denote where BTx642 increased the magnitude of the trait. Field 2009 QTL are shown in black and field 2010 are in red. All QTL are shown at one LOD below the QTL peak.

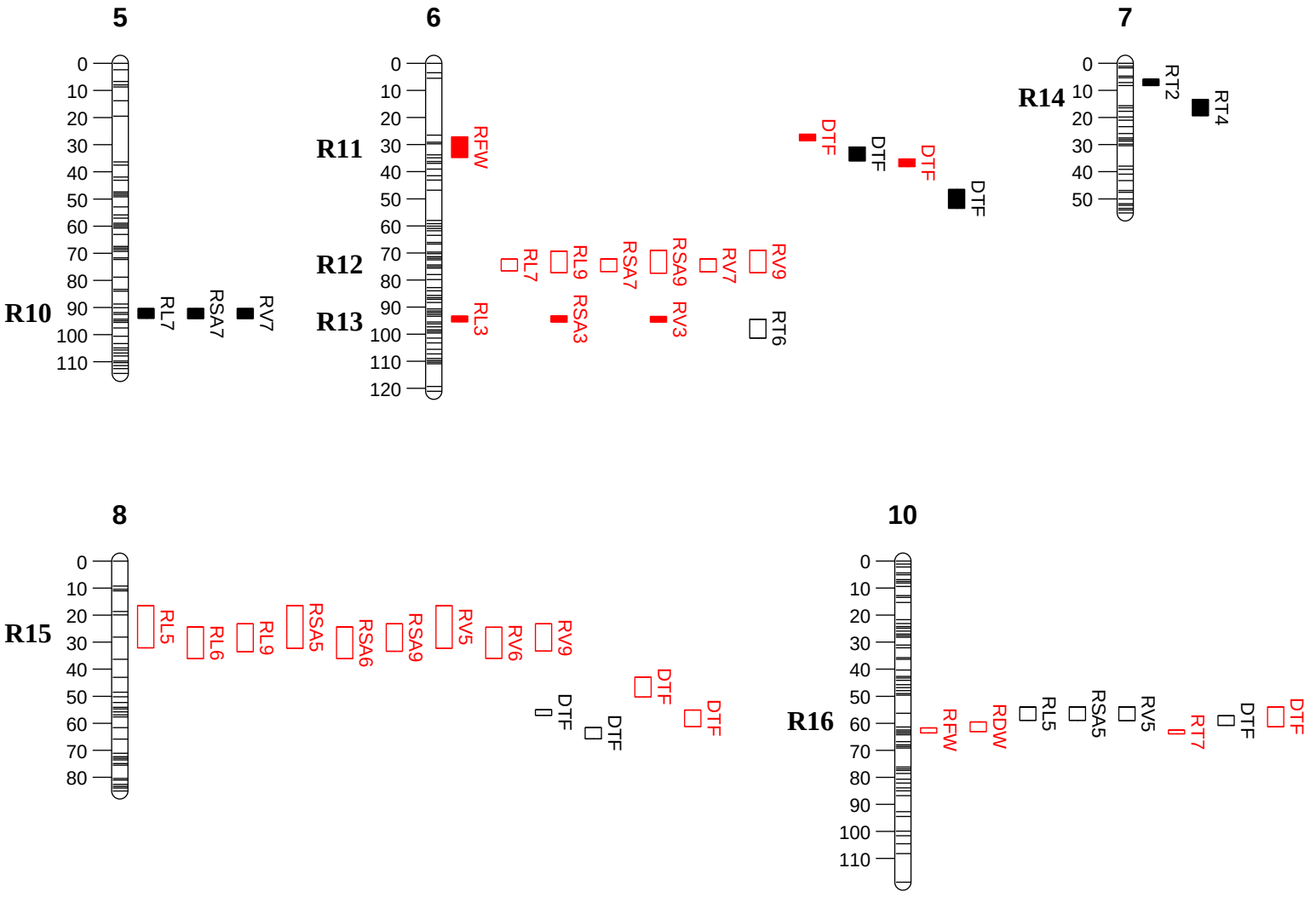


Figure 8.1, continued.

Six QTL regions were identified in 2009 and 12 regions were identified in 2010. Two regions, R4 on LG-01 and R16 on LG-10, were found to contain co-localized QTL from 2009 and 2010. In general, root length, surface area, and volume QTL clustered by size diameter class. Several size classes were often found co-aligned. For example, regions R1, 6, 10, 12, 15, and 16 contained size parameter QTL for roots 2.0 and greater in diameter (≥Class 5), and regions R2, 3, 5, 7, and 14 contained QTL for roots less than 2.5 mm in diameter (≤Class 5). Four regions were identified that contained root size QTL for all root diameters: R4, 8, 9, and 13. Root total volume QTL were found overlapping large diameter root QTL regions on LG-02 and LG-04; root total surface area QTL were detected co-localized with all root size QTL in region R7, and root total number of tips were found in regions R5 and R8, co-aligned with QTL for small root diameter classes and all root diameter classes, respectively.

Discussion

The size and function of the sorghum root system will have an important impact on this plant's capacity to access water and nutrients from the soil especially under water limiting conditions. However, analysis of root systems is challenging especially in larger field grown plants because roots are distributed in the soil profile and must be excavated for analysis or analyzed through indirect imaging methods. In this study, the root systems of BTx642, RTx7000 and RILs derived from these genotypes were excavated from the field at 52 DAE in 2009 and 2010, washed, and imaged to assess variation in overall root system size and complexity. The analysis was performed on plants at 52 DAE because this is approximately mid GS2 and about the time when water

deficit begins in environments where plants will be exposed to post-flowering terminal drought stress. Therefore differences in the root systems of BTx642 and RTx7000 at 52 DAE could influence the post-flowering drought response of these genotypes.

Total root length, surface area, and volume and the number and dimensions of the root hairs, nodal roots, and lateral roots are important metrics of root systems. These root trait metrics were measured using a scanning system that quantified the length and diameter of each root and root hair and recorded the number of tips of roots and root hairs of different diameter as well as the surface area and volume of each root diameter class. Root hairs had the smallest diameter (0-0.5 mm) and length (<1.5 mm) (root diameter size class 1). The number of root hairs per root system was very large (~3-5,000 for parental lines) as expected. Roots with the largest diameter (> 2 mm) corresponded to the nodal roots present at this stage of development (root diameter size classes 5-9). Roots with intermediate size diameter correspond mostly to lateral roots of varying length (root diameter size classes 2-4).

BTx642 and RTx7000 root systems at 52 DAE contained similar amounts of dry weight. However, in 2009 the RTx7000 root system had greater total surface area and volume, a difference that was primarily due to longer and larger nodal roots. The sum of the length of nodal roots (root classes 5-9) in RTx7000 was ~30% greater than BTx642 and there was a corresponding increase in nodal root surface area and volume in RTx7000. BTx642 showed somewhat elevated numbers of root hairs and root forks resulting from root hair generation relative to RTx7000. Analysis of roots of the RIL population derived from BTx642 and RTx7000 revealed transgressive segregation for all

root traits analyzed with 1.5-11.0 fold variation among RILs depending on the specific root trait analyzed.

Overall broad sense heritability of root traits ranged from 0.30-0.40 for most traits. Residual variation was high, ranging from ~40-80% across traits. One source of this variation could be technical in that more advanced scanning technology was used in 2010, increasing resolution (dpi) by one-third while at the same time increasing the scanning bed by 2.4 fold relative to 2009. Through such measures, much higher quality images were generated in 2010 because the increased scanning area allowed the root system to be dispersed more uniformly (fewer roots overlapped each other). However, considering that root weight also showed ~50-60% residual variation, and weight is independent of scanning, other sources of variation were probably present. An additional source could arise from the plastic nature of root growth, meaning that roots are able to make morphological changes based on characteristics of the soil they are growing in. For example, in the phenology study root systems of the parents reached ~1.5 m by 36 DAE, whereas plants growing in the same soil type in the field reached a depth of only ~0.3 m by 52 DAE. This difference is due to many factors, but an important one with respect to soil is density. Much more variation in soil compaction was observed under field conditions than in the phenology study. Even within a given row, differences in soil compaction were evident, as the ease of root extraction from the soil was variable. Residual effects may be reduced through additional studies in which more reps and more plants per rep are assayed.

QTL analysis of root traits in the RIL populations identified 16 regions of the sorghum genome that modulate root number, length, surface area, volume, branching, and/or weight (Fig. 8.1). This is a minimum estimate of the number of alleles in this population that modulate root traits due to small population size and environmental effects. BTx642 alleles increased the root trait measured in seven of the QTL and RTx7000 alleles increased the traits measured in nine QTL. Five QTL regions modulated size parameters associated with small roots whereas six QTL modulated traits associated with large roots. Four QTL for total root weight, one QTL for root branching (forks), and two QTL for root tip number were identified. BTx642 alleles were responsible for increased root hair (root diameter class 1) number and total root tip number, consistent with higher root hair number in BTx642 compared to RTx7000 in 2009. In contrast, in 2010 a new root tip number region, R8, was detected in which RTx7000 alleles were detected, consistent with the higher total root tip number seen in RTx7000 relative to BTx642 in 2010. Several QTL affected the number and/or size of roots of similar diameter classes. For example, region R13 modulated the number and size of root diameter class 3. This diameter root may correspond to lateral roots and an increase in number or length of these roots would increase the overall density of the root system per unit soil volume. Region R6 affects root size classes 5-8, and R10 and R1 affect root size classes 7 and 8, respectively. The observed partitioning of regulation by nodal root size class may reflect differential control during development because the smallest nodal roots (classes 5-6) may be formed at an earlier stage of development compared to the largest nodal roots (class 9).

Interestingly, relative to the shoot size parameters discussed in previous chapters, root size parameter QTL, while still numerous, had comparatively fewer associations with flowering time QTL (Fig. 8.1). The overlap between a QTL encoding an allele from BTx642 in 2009 (R16) that increases the length and surface area of nodal root class 5 and a maturity allele from BTx642 that delays flowering provides one tentative connection between plant development phase and root growth traits. A second connection was seen in 2010 between maturity and root weight in which a delay in flowering caused by RTx7000 alleles (R11) or BTx642 alleles (R16) was associated with an increase in root weight by the same respective alleles. The possible association between root traits and other plant traits characterized in this study will be explored in the next chapter.

CHAPTER IX

ANALYSIS OF TRAIT VARIATION AND QTL IDENTIFIED THROUGH THE STUDY OF BTx642 AND RTx7000

Introduction

Analysis of the growth and development of BTx642 and RTx7000 revealed numerous differences in phenology, leaves, stems, and roots that had the potential to modulate the accumulation of plant biomass, grain yield, and response to water deficit. Traits that differed in these genotypes included time to anthesis, shoot biomass, stalk biomass, stem biomass, stalk and stem length, individual leaf weight, leaf area, leaf width, leaf length, total leaf area, and root system architecture. In general, trait variation was specific to leaves, stems, roots, or panicles. On the other hand, variation in traits such as flowering time influenced several other traits (leaf size, for example).

Trait segregation was analyzed in a RIL population derived from BTx642 and RTx7000. A genetic map was generated using DNA from 90 RILs to facilitate analysis of the genetic basis of variation of traits segregating in the population. The genetic map was comprised of 566 DG markers. The map spanned 1130 cM and ~583 Mbp with an average of 2.0 cM between markers and ~526 Kbp/cM. Segregation distortion was observed on six of the linkage groups, with the most severe distortion occurring on linkage groups three and ten.

Traits were analyzed in the BTx642 x RTx7000 RIL population at anthesis and at grain maturity in field conditions in 2008-2010 and at anthesis in greenhouse conditions. The traits analyzed during this study were time to anthesis, stalk length, shoot biomass

(leaf, stalk, stem, leaf sheath, and combined), leaf size (length, width, area, and total area), root size (length, surface, and volume, and number of root tips and forks), root biomass, and panicle biomass. Extensive transgressive segregation in the RIL population was observed for most traits. This provided an opportunity to search for potential associations among component traits that affected leaves, stems, or roots and output traits such as biomass accumulation and grain yield. Potential trait associations were analyzed through QTL analysis. In general, broad sense heritability was sufficient across environments to allow QTL to be identified with high confidence. Under field conditions broad sense heritability was ~0.77-0.90 for shoot biomass, ~0.66-0.68 for panicle weight at grain maturity, ~0.55-0.86 for leaf size, and ~0.30-0.40 for root size. Overall, QTL were identified on 90% of the linkage groups of the sorghum genome. One to seven QTL were found to modulate each trait analyzed in a specific environment and stage of development.

In total, 72 different traits were analyzed in the RIL population and 477 QTL were mapped across four environments (Fig. 9.1). In many instances, QTL affected several related traits associated with a single type of organ system (for example, different types of root traits) but QTL infrequently had an impact on traits associated with different organs (for example, roots vs. leaves). Several QTL were identified in only one environment. Some of this specificity of QTL detection by organ system was expected due to small population size that limited detection of QTL with small effects. However, the specificity observed is consistent with independent genetic control of traits associated with leaves, stems and roots.

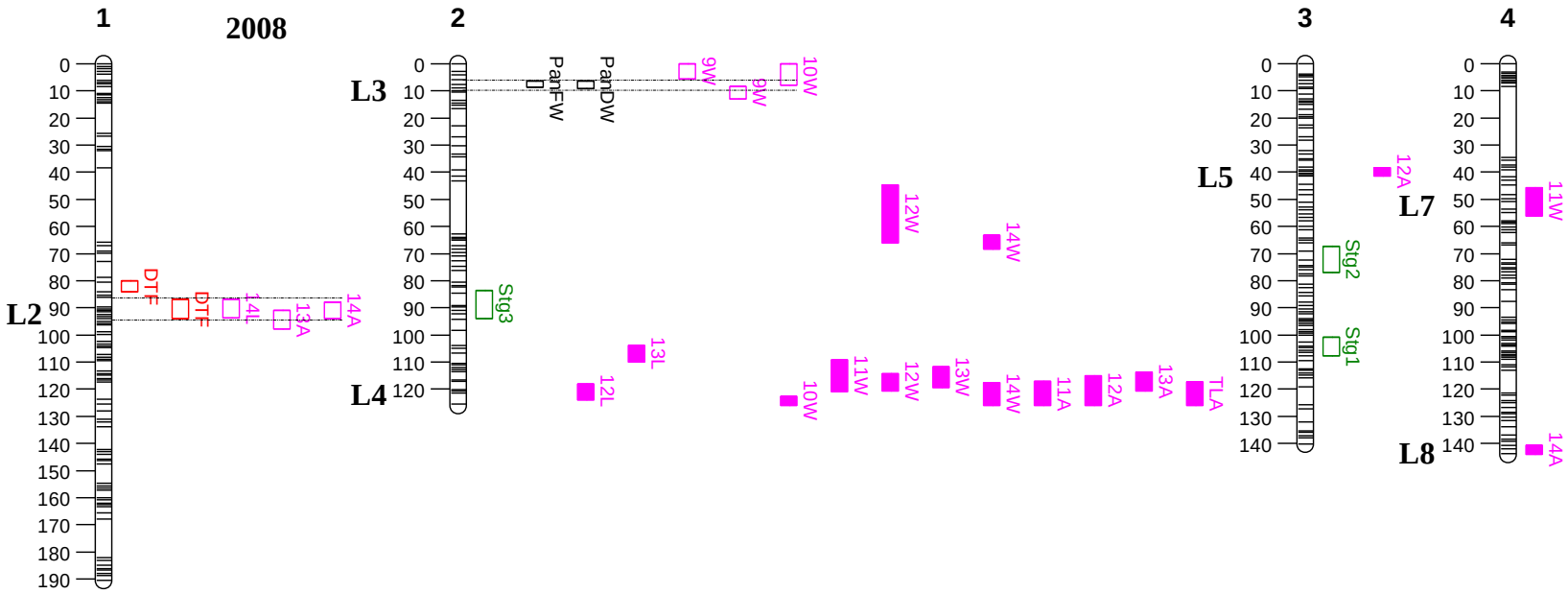
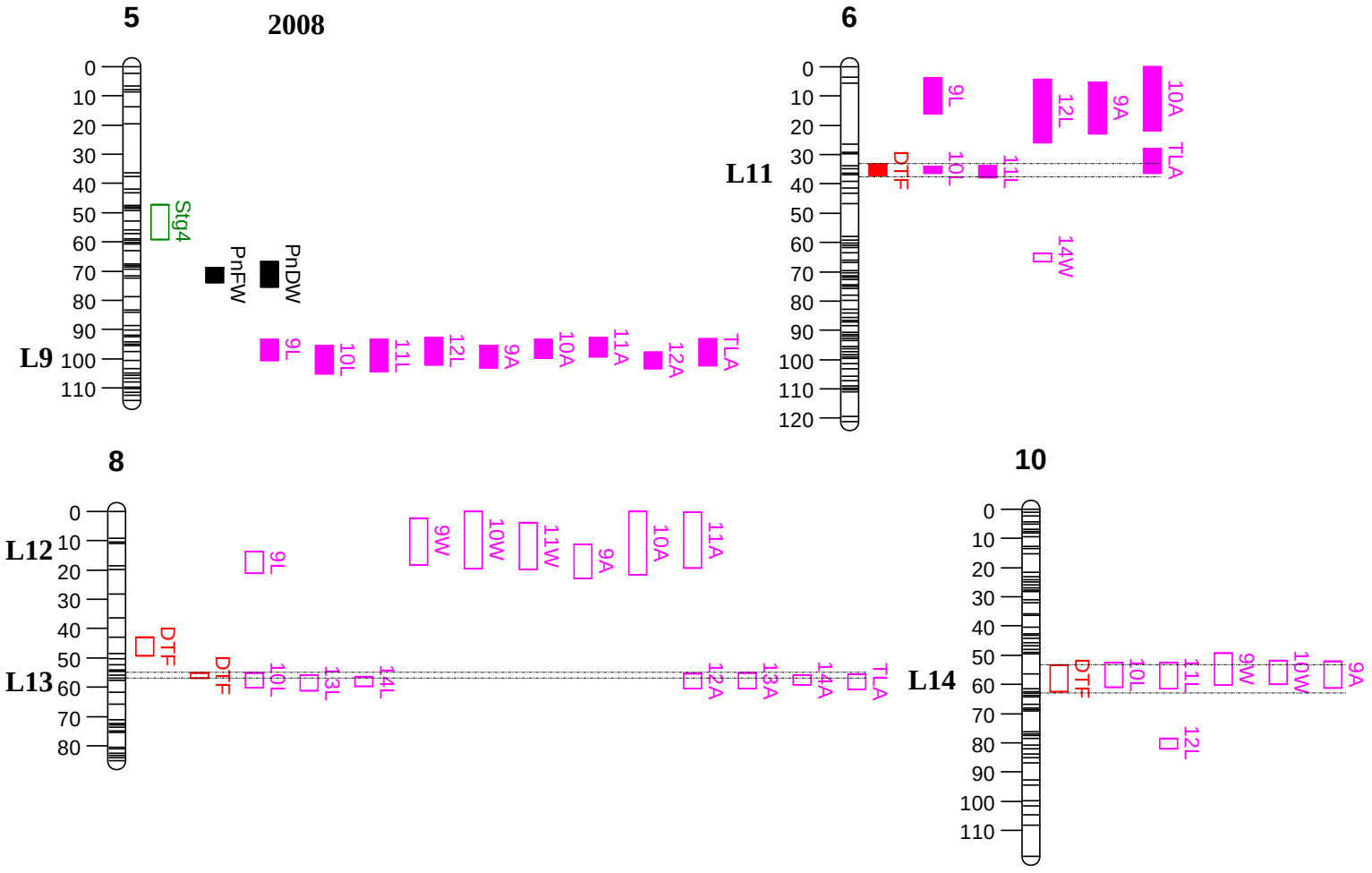


Figure 9.1. Quantitative trait loci (QTL) summation for field 2008-2010 and greenhouse conditions. QTL are shown for leaves 9-14 leaf length (L), width (W), area (A), and total leaf area (TLA), shoot (S), stalk (STK), stem (STM), leaf (L), leaf sheath (LS), and panicle (Pan) fresh weight (FW) or dry weight (DW), flowering time (DTF), stalk length (Dw), and stay green loci 1-4 (Stg1-4). QTL are also shown for root length (RL), root surface area (RSA), root volume (RV), number of root tips (RT), number of root forks (FKS), and root fresh (RFW) or dry weight (RDW). Roots were divided into classes based on root diameter, starting at 1, (0.0-0.5 mm) and stepping incrementally by 0.5 mm to class 9 (≥4.5 mm). Traits with a "T" denote summation of classes for the respective trait. Filled bars denote QTL where RTx7000 increased the magnitude of the trait; unfilled bars denote where BTx642 increased the magnitude of the trait. Flowering time QTL are shown in red, stalk length in blue, stay green in green, panicle weight in black, shoot biomass parameters in cyan, leaf size parameters in fuchsia, and root size parameters in bright green. All QTL are shown at one LOD below the QTL peak.



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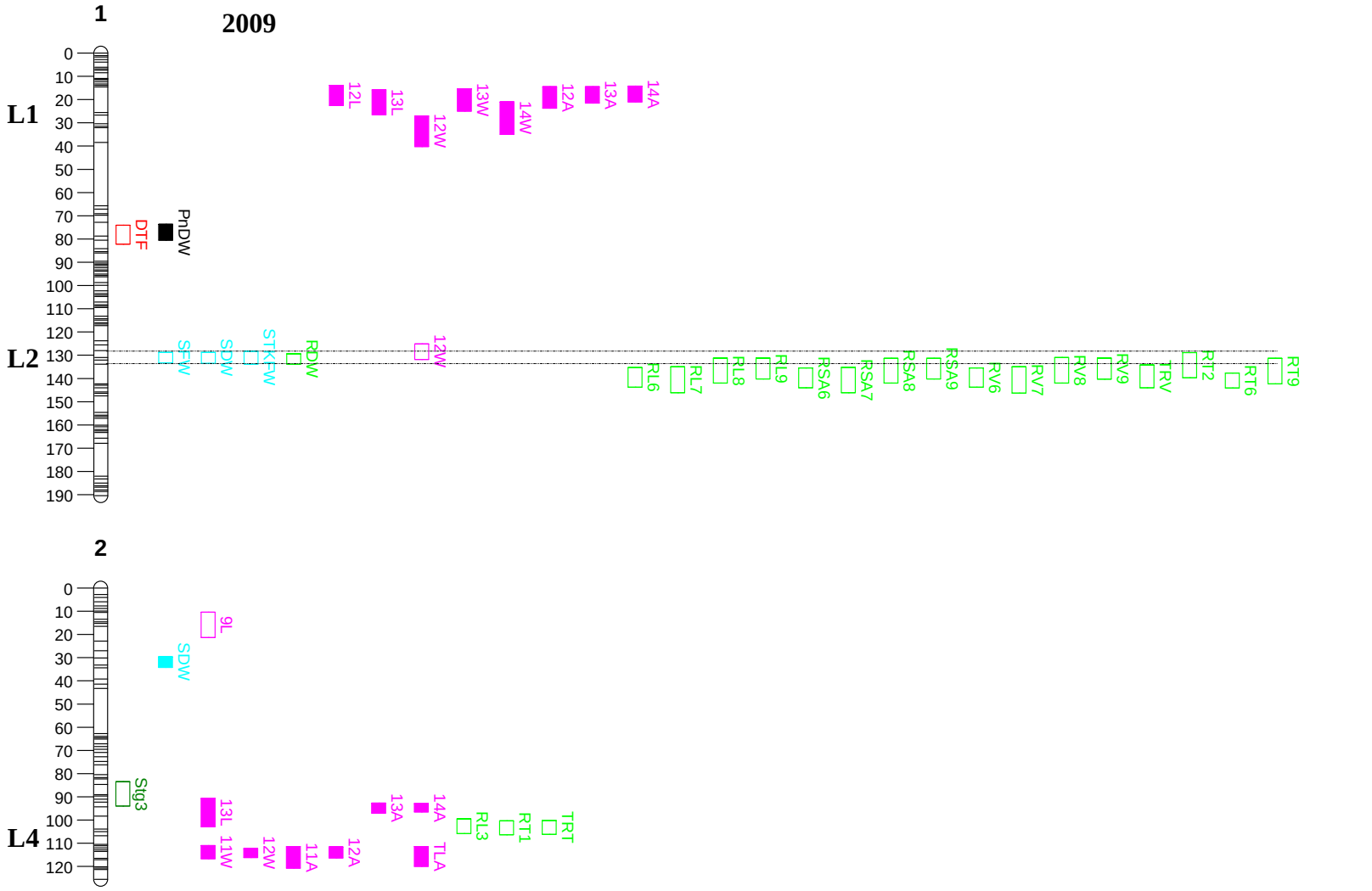

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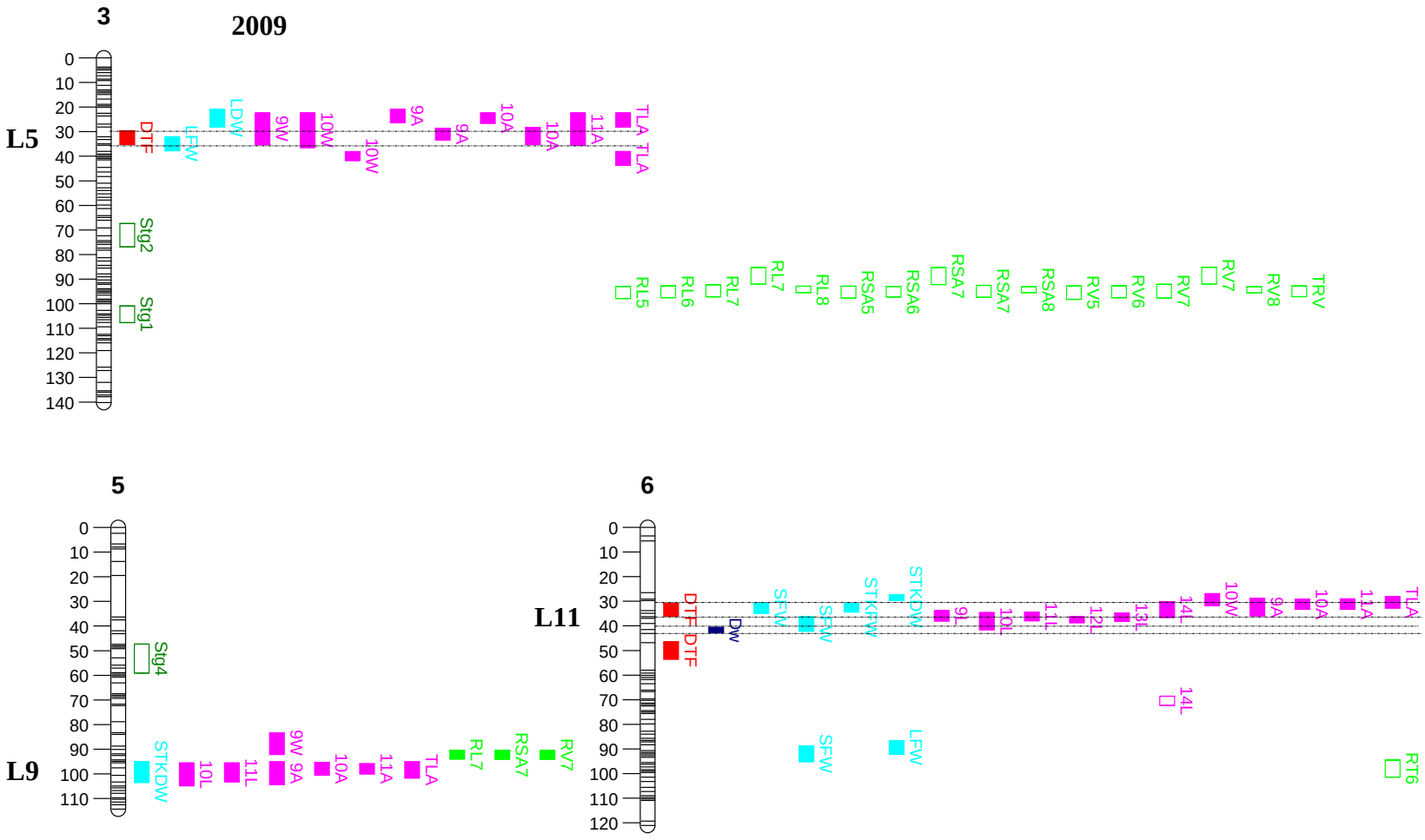

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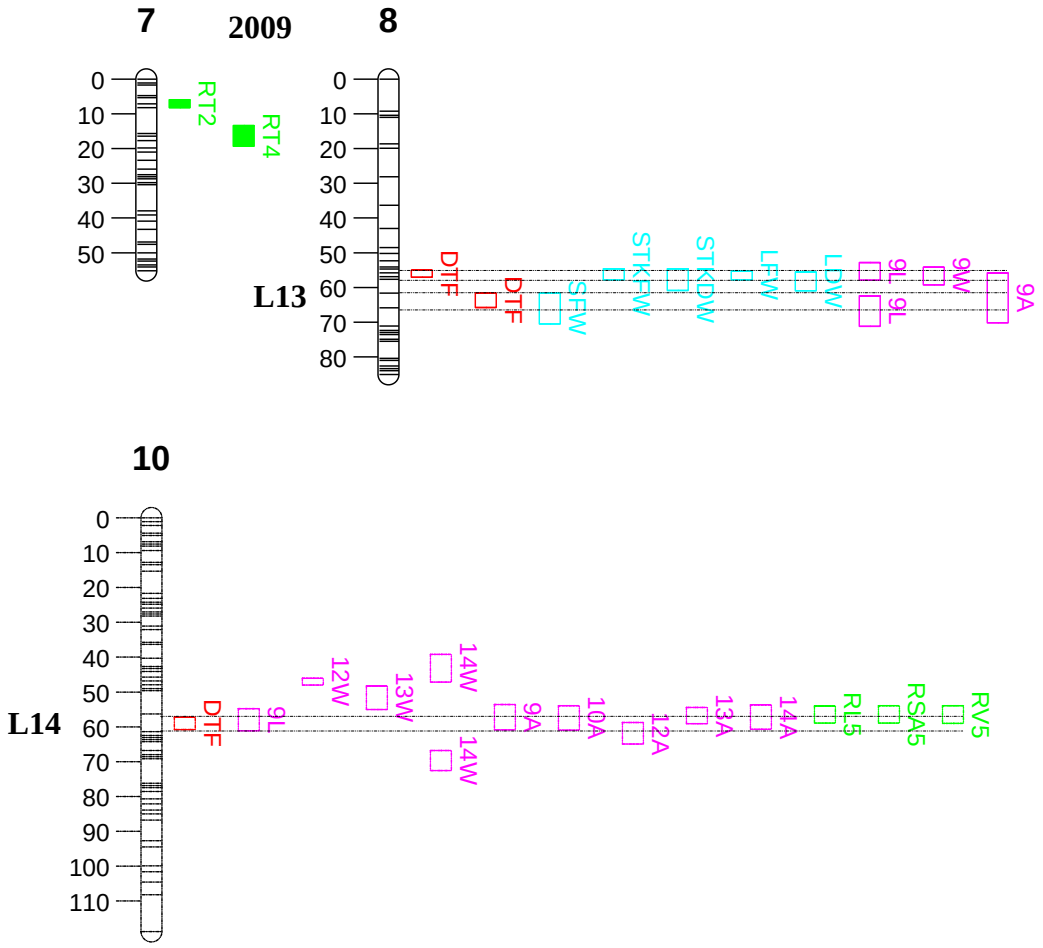

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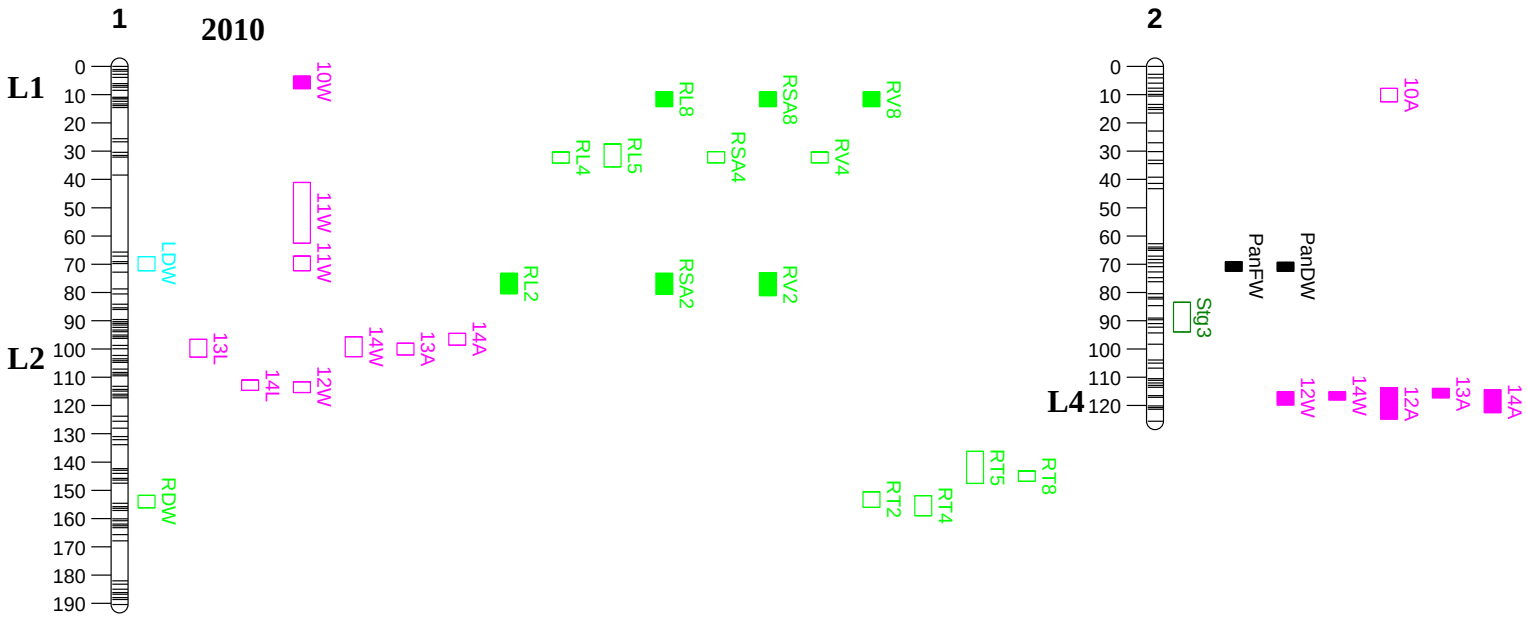

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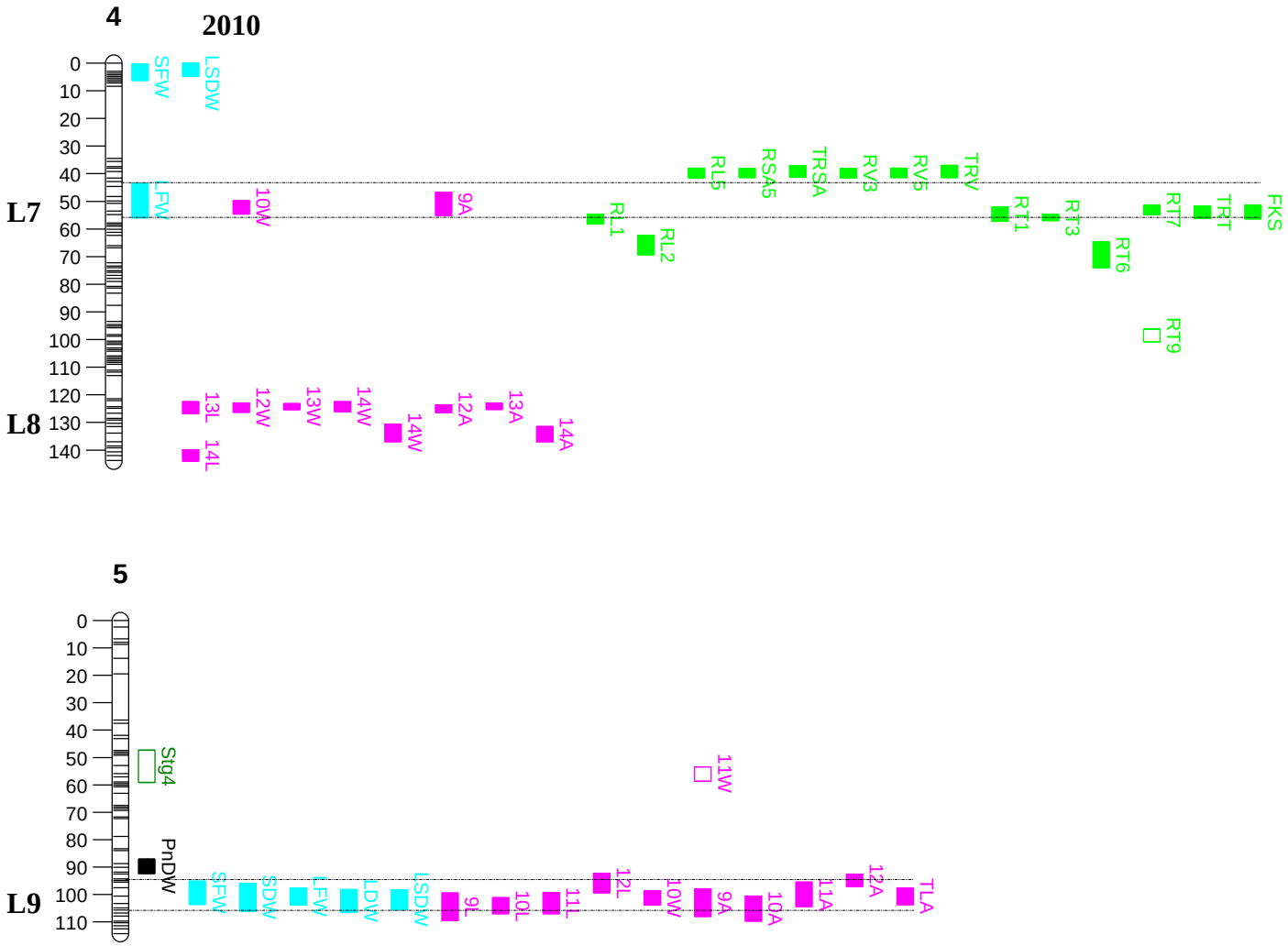

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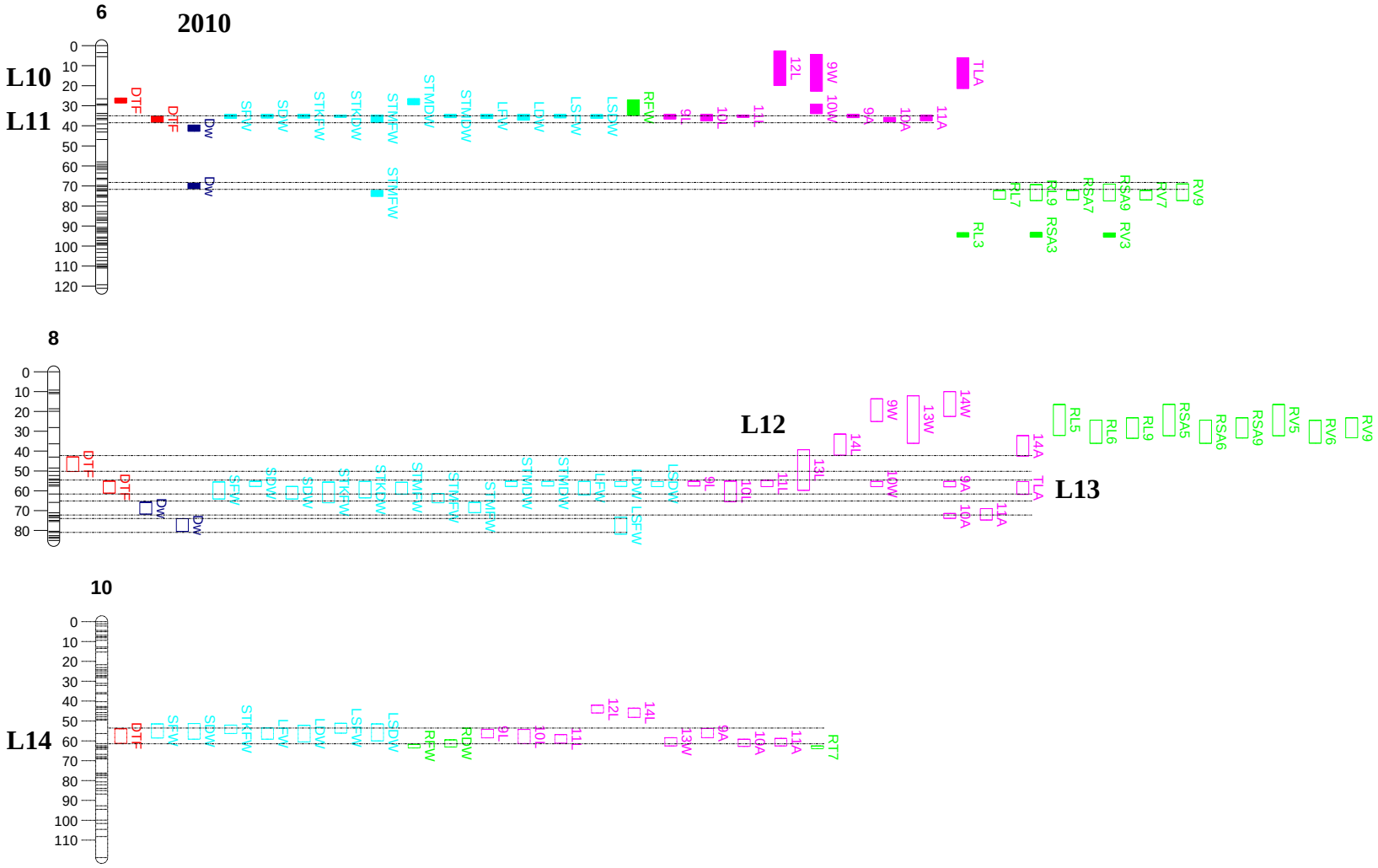

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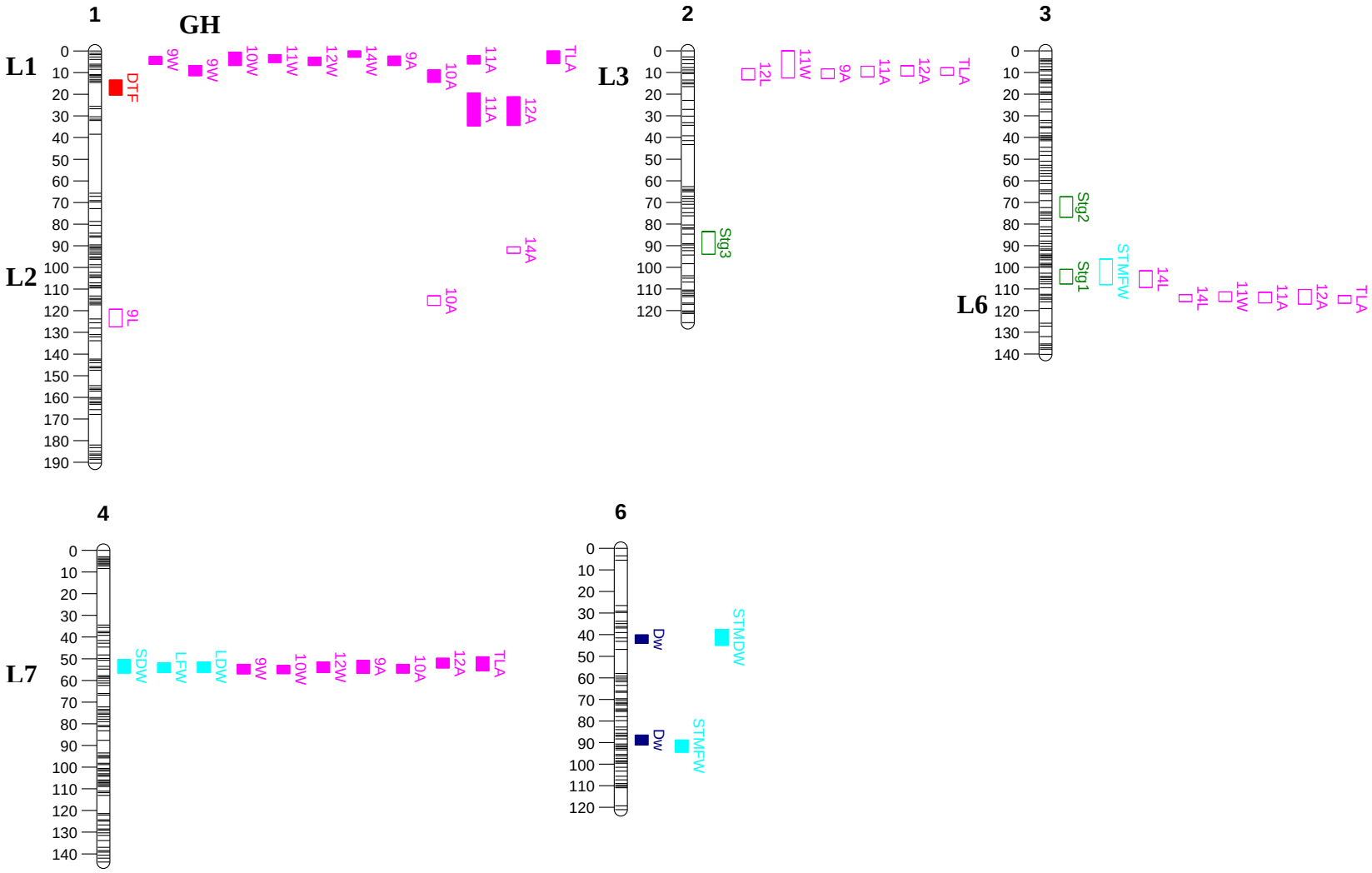

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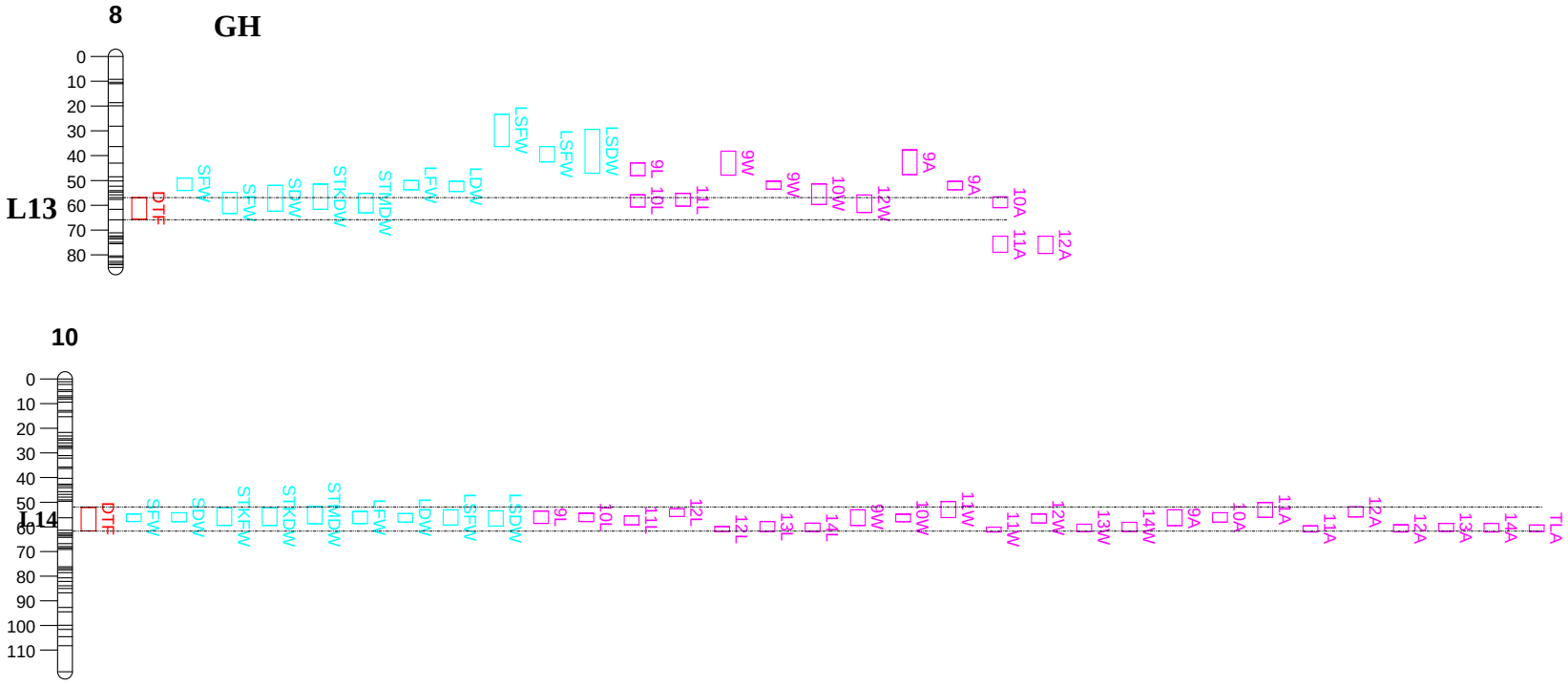

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Flowering time QTL were mapped to six regions of the genome in greenhouse pots and field conditions. Two of six loci were identified under field and short day greenhouse conditions (LG-08 and LG-10). Flowering time QTL were often co-located with QTL for several shoot and leaf traits, consistent with flowering time having an influence on leaf number, size, and overall shoot size. Stalk length QTL were detected in six regions of the genome. The stem/stalk length QTL at ~41 cM on LG-06 that corresponds to *Dw2* was identified in both field and greenhouse conditions. The overlap between stalk length and other QTL identified was less relative to flowering time; stalk length was correlated mainly with shoot biomass parameters (2009: LG-06, 2010: LG-06, LG-08, greenhouse: LG-06). As the plant grows in height, shoot biomass increases together with the expanding stalk/stem, thus providing an explanation for the association seen between changes in shoot biomass and stalk length. QTL for shoot biomass were dispersed across 12 regions of the genome, leaf size 14 regions, and root size 16 regions.

Results and Discussion

Association Between Shoot Biomass and Leaf and Root Size Parameters

QTL for leaf size were often co-located with QTL for shoot weight parameters (shoot, stalk, stem, leaf, and leaf sheath weight) because leaf weight contributes significantly to shoot biomass at anthesis (Fig. 9.1). In 2009, overlapping QTL were found in regions L2, 5, 9, 11, and 13; in 2010 regions L7, 9, 11, 13, and 14 contained co-localized QTL, and in the greenhouse regions L7, 13, and 14 showed alignment between leaf size and shoot biomass parameters. Regions L13 and 14 were detected in all three environments, and region L11 was detected in field conditions in 2009-2010. All three

of these loci were affected by flowering time, which seems reasonable because delayed flowering increases the duration of leaf growth which in turn could increase the leaf's photosynthetic capacity, resulting in more assimilate storage in leaves and stalks. One additional region, L9, was also detected in field conditions in 2009-2010 in a region not affected by flowering time.

Leaf size and shoot biomass traits were also found in regions independent of one another and independent of flowering time, showing that these two groups of traits are not entirely co-regulated, nor are they entirely regulated by flowering time. In 2009, leaf size-only QTL fitting these criteria were found in two regions (L1 and 4), in 2010 six regions (L1, 2, 4, 8, 10, 12), and in the greenhouse three regions (L1, 3, and 6). Shoot biomass-only QTL were also found in these three environments (2009: LG-02 and 6, 2010: LG-01 and 6, and greenhouse: LG-06).

Eleven QTL that modulate total leaf area (TLA) were detected in the studies: four in 2008, four in 2009, three in 2010, and five in greenhouse pots. In three cases total leaf area QTL were observed across environments: L4 in 2008-2010, L11 in 2008-2009, and L13 in 2008 and 2010. QTL for TLA co-localized with QTL that affected the size of specific leaves in all cases. In general QTL for TLA were associated with RTx7000 alleles that increased the size of upper leaves. This is consistent with the finding that the upper leaves of RTx7000 are larger than BTx642 leaves at anthesis in plants grown under field conditions. Additionally, it seems reasonable to expect that total leaf area QTL would be associated with individual leaf size QTL of the upper leaves since the larger upper leaves contribute more to TLA than smaller leaves.

Root size QTL had little correspondence with shoot weight parameters (shoot, stalk, stem, leaf, and/or leaf sheath weight), suggesting that root size and shoot parameters are regulated by different pathways in development (Fig. 9.1). In 2009 minimal overlap was seen between shoot biomass and root size QTL on LG-01; in 2010 overlap was seen on LG-04 and LG-06.

Association Between Panicle Biomass and Leaf and Root Size Parameters

Two leaf size QTL overlapped panicle weight but were not coincident with shoot weight QTL (2008, LG-02). This may indicate that these loci are involved with producing assimilate used directly in panicle production as opposed to first being routed to stem and leaf sinks. QTL for total leaf area were not associated with variation in panicle weight. This implies that the plant has a canopy of sufficient size and photosynthetic activity to supply the developing panicle and that carbon fixation does not limit biomass accumulation in the panicle. QTL for panicle biomass at grain maturity did not coincide with QTL that modulate root size or other root traits measured at 52 DAE. This suggests that root system size also does not limit panicle biomass accumulation under these conditions. However, it is possible that the size and architecture of the root system changes from 52 DAE to grain maturity. Therefore it will be necessary to assay root system architecture at grain maturity to obtain a more complete understanding of the relationship between root system size and grain yield.

In 2009 a panicle dry weight QTL overlapped with a flowering time QTL on LG-01, making it difficult to discern whether effects at this locus are influenced by the

length of the vegetative period, which is directly affected by the timing of shoot apical meristem differentiation.

Association Between Stay Green and Shoot Biomass and Leaf and Root Size Parameters

Xu et al. (2000) detected four stay green QTL in the BTx642/RTx7000 RIL population under terminal drought conditions. *Stg1* located on LG-03, coincides with a QTL for stem weight at anthesis in greenhouse conditions. Stay green is correlated with reduced stalk lodging. Increased stem biomass at anthesis could reduce lodging if the biomass is allocated to structural carbohydrate. Alternatively, if the increased stem biomass in plants containing the BTx642 allele is due to accumulation of non-structural carbohydrate, then this could serve as a source of carbon for grain filling under conditions of water deficit. However, environmental effects between greenhouse and field conditions were significant ($P<0.001$), explaining 70% of the total variation and broad sense heritability was low at 0.15 (Chapter VI). Therefore, the stem weight QTL observed only in the low light environment of the greenhouse is probably not relevant to the expression of the stay green phenotype under water-limiting field conditions.

QTL that modulate leaf size under water sufficient conditions had minimal overlap with *Stg1-4*. In 2010 a QTL for leaf 11 width coincided with *Stg4* and in greenhouse conditions a QTL for leaf 14 length coincided with *Stg1*. It is possible that leaf area development in the RIL population is modulated by stress and that this contributes to the stay green trait. It has also been proposed that BTx642 produces thicker leaves than RTx7000 that contain the same amount (or more) of photosynthetic machinery as RTx7000 in order to compensate for the reduced surface area. It is also

possible that BTx642 has a lower stomata density and/or a different type of stomatal regulation than RTx7000 under water-limiting conditions. Neither of these facets has been explored in this study, but would be of future interest since both of these traits could influence storage of assimilate in the stem. QTL that modulate root size and architecture under water sufficient conditions also did not coincide with *Stg1-4*. It is possible that root size as well as other shoot QTL were not detected overlapping stay green loci because different regulation is at play in a well-watered environment as opposed to a water-limited environment. Further studies will need to be conducted to explore this question.

Allele Contrast Analysis

Regions of the genome containing co-localizing QTL were examined for evidence of pleiotropy through using allele contrast analysis. Briefly, in regions of the genome containing co-localized QTL, a marker common to all QTL in the region and exhibiting high linkage to the peak of all QTL was identified. Subsequently, RILs were placed in two classes based on the parental genotype of the RIL at the selected locus. Corresponding trait values for each RIL of the respective genotype were averaged and the means of the two groups compared via a *t*-test to identify significant differences between the parental alleles. Regions in which trait means were significantly higher for the same parental allele across QTL provided evidence of pleiotropy.

As shown previously, several regions of the genome were identified containing co-localized QTL. With respect to root size QTL, a region on LG-01 centered on marker DG65 was identified under field conditions in 2009. This region contained QTL for root

length, surface area, volume, and number of root tips for large diameter roots (>3.0 mm). As shown in Table 9.1, the BTx642 allele contributed to significantly higher ($P<0.001$) trait values (1.2-1.6 times greater) than the RTx7000 allele in all 15 QTL, providing evidence of pleiotropy at this locus. For leaf size parameters, a QTL region was identified on LG-02 at marker DG139, at which QTL for leaves 12-14 width and area under field conditions in 2010 were found co-aligned. Allele contrast analysis revealed that the RTx7000 allele trait values were significantly greater ($P<0.001$) than BTx642 allele trait values for all five leaf size QTL at this locus (Table 9.2).

Leaf size and shoot biomass parameter QTL were often detected coinciding. Linkage group five at marker DG356 provides an example of QTL for leaves 9-11 length, width, and area QTL coinciding with QTL for leaf, leaf sheath, and shoot weight. In Table 9.3 it is shown that the RTx7000 allele trait means are significantly greater than the BTx642 allele trait means in all 13 QTL in this region, providing evidence of pleiotropy.

Flowering time QTL were also frequently found co-located with QTL for leaf size and shoot biomass. At marker DG375 on LG-06, allele contrast analysis revealed that the RTx700 allele trait means were significantly greater ($P<0.001$) than the BTx642 allele trait means in all 15 QTL at this locus under field conditions in 2010. The RTx7000 allele trait means ranged from 1.1-1.3 times greater than the BTx642 allele trait means for leaf size and ranged from 1.2-1.9 times greater for shoot biomass (Table 9.4).

Table 9.1. Allele contrast analysis for root size parameters under 2009 field conditions at marker DG65 in the BTx642 x RTx7000 RIL population.

Trait[†]	BTx642 Mean (SE)[‡]	RTx7000 Mean (SE)[‡]	P Value[§]	Mean Difference[¶]	Fold Difference[#]
RL6	38.6 (1.3)	32.9 (1.6)	6.22E-03	5.7	1.2
RL7	27.8 (1.1)	22.7 (1.2)	2.22E-03	5.1	1.2
RL8	19.0 (0.8)	15.4 (0.9)	2.40E-03	3.6	1.2
RL9	13.8 (0.6)	11.1 (0.7)	3.58E-03	2.7	1.2
RSA6	33.2 (1.1)	28.2 (1.4)	5.97E-03	5.0	1.2
RSA7	28.2 (1.1)	23.1 (1.2)	2.21E-03	5.1	1.2
RSA8	22.4 (0.9)	18.1 (1.0)	2.43E-03	4.3	1.2
RSA9	18.4 (0.8)	14.8 (0.9)	3.55E-03	3.6	1.2
RV6	2.3 (0.08)	1.9 (0.09)	5.74E-03	0.4	1.2
RV7	2.3 (0.09)	1.9 (0.1)	2.20E-03	0.4	1.2
RV8	2.1 (0.09)	1.7 (0.09)	2.47E-03	0.4	1.2
RV9	2.0 (0.09)	1.6 (0.09)	3.53E-03	0.4	1.3
Tips2	81.6 (2.5)	69.8 (2.7)	4.87E-03	11.8	1.2
Tips6	3.7 (0.2)	2.8 (0.2)	1.25E-03	0.9	1.3
Tips9	1.3 (0.09)	0.8 (0.07)	5.84E-03	0.5	1.6

[†]Root size diameters were divided into 0.5 mm classes, starting at class 1 including roots of diameter up to 0.5 mm and stepping in 0.5 increments to size diameter class 9. Traits included root length (RL) in cm, root surface area (RSA) in cm^2, root volume (RV) in cm^3, and the number of root tips (Tips).

[‡]Standard error.

[§]*t*-test calculation of significance level at which parental allele means are different.

[¶]Diffference between the means of the high parent allele (BTx642) and the low parent allele (RTx7000).

[#]High parent allele (BTx642) mean divided by the low parent allele (RTx7000) mean.

Table 9.2. Allele contrast analysis for leaf size parameters under 2010 field conditions at marker DG139 in the BTx642 x RTx7000 RIL population.

Trait[†]	BTx642 Mean (SE)[‡]	RTx7000 Mean (SE)[‡]	P Value[§]	Mean Difference[¶]	Fold Difference[#]
L12W	9.5 (0.1)	10.5 (0.1)	1.18E-04	1.0	1.1
L12A	401.6 (7.6)	455.6 (10.1)	1.70E-03	54.0	1.1
L13A	352.8 (7.3)	402.7 (8.8)	1.54E-03	49.9	1.1
L14W	7.5 (0.1)	8.3 (0.1)	9.34E-04	0.8	1.1
L14A	198.9 (5.7)	234.9 (6.3)	2.05E-03	36.0	1.2

[†]Leaf size parameters of leaves 12-14 width (W) in cm, length (L) in cm, and area (A) in cm^2.

[‡]Standard error.

[§]*t*-test calculation of significance level at which parental allele means are different.

[¶]Diffference between the means of the high parent allele (RTx7000) and the low parent allele (BTx642).

[#]High parent allele (RTx7000) mean divided by the low parent allele (BTx642) mean.

Table 9.3. Allele contrast analysis for leaf size and shoot biomass parameters under 2010 field conditions at marker DG356 in the BTx642 x RTx7000 RIL population.

Trait[†]	BTx642 Mean (SE)[‡]	RTx7000 Mean (SE)[‡]	P Value[§]	Mean Difference[¶]	Fold Difference[#]
L9L	63.4 (1.7)	69.4 (1.7)	1.81E-02	6.0	1.1
L9A	309.6 (14.8)	363.9 (16.8)	2.30E-02	54.3	1.2
L10L	68.0 (1.4)	73.7 (1.4)	8.97E-03	5.7	1.1
L10W	9.0 (0.2)	9.7 (0.2)	2.50E-02	0.7	1.1
L10A	358.7 (12.9)	409.8 (14.3)	1.50E-02	51.1	1.1
L11L	71.6 (1.1)	76.2 (1.1)	6.13E-03	4.6	1.1
L11A	397.1 (10.1)	441.9 (11.4)	7.33E-03	44.8	1.1
TLA	2283.4 (61.5)	2584.4 (75.2)	6.69E-03	301.0	1.1
LFW	66.1 (2.6)	79.2 (3.9)	1.26E-02	13.1	1.2
LDW	15.9 (0.6)	18.8 (0.9)	1.76E-02	2.9	1.2
LSDW	13.3 (0.5)	15.5 (0.7)	2.17E-02	2.2	1.2
SFW	287.0 (10.7)	333.6 (14.5)	1.93E-02	46.6	1.2
SDW	58.4 (2.3)	68.4 (2.9)	1.16E-02	10.0	1.2

[†]Leaves 9-11 length (L) in cm, width (W) in cm, and area (A) in cm^2, and leaf (L), leaf sheath (LS), and shoot (S) fresh (FW) and dry (DW) weight in g.

[‡]Standard error.

[§]*t*-test calculation of significance level at which parental allele means are different.

[¶]Diffference between the means of the high parent allele (RTx7000) and the low parent allele (BTx642).

[#]High parent allele (RTx7000) mean divided by the low parent allele (BTx642) mean.

Table 9.4. Allele contrast analysis for flowering time and leaf size and shoot biomass parameters under 2010 field conditions at marker DG375 in the BTx642 x RTx7000 RIL population.

Trait[†]	BTx642 Mean (SE)[‡]	RTx7000 Mean (SE)[‡]	P Value[§]	Mean Difference[¶]	Fold Difference[#]
DTF	64.6 (0.7)	68.1 (0.7)	3.59E-04	3.5	1.1
L9L	60.9 (1.5)	72.2 (1.6)	2.68E-06	11.3	1.2
L9A	291.3 (13.5)	385.5 (15.9)	2.35E-05	94.2	1.3
L10L	66.1 (1.3)	75.7 (1.3)	2.82E-06	9.6	1.1
L10A	345.7 (12.7)	425.8 (13.1)	2.94E-05	80.1	1.2
L11L	70.9 (1.2)	76.9 (0.9)	1.77E-04	6.0	1.1
L11A	393.8 (11.2)	446.3 (10.2)	6.18E-04	52.5	1.1
LFW	65.2 (2.8)	80.7 (3.7)	1.13E-03	15.5	1.2
LDW	15.5 (0.6)	19.3 (0.9)	8.30E-04	3.8	1.2
STMFW	57.5 (4.1)	112.1 (7.2)	5.10E-09	54.6	1.9
STMDW	11.4 (0.8)	21.1 (1.5)	2.90E-07	9.7	1.9
STKFW	148.2 (6.4)	226.6 (10.6)	1.27E-08	78.4	1.5
STKDW	28.5 (1.3)	42.9 (2.2)	4.35E-07	14.4	1.5
SFW	269.1 (9.4)	354.6 (13.6)	1.99E-06	85.5	1.3
SDW	56.4 (2.0)	70.9 (2.9)	1.01E-04	14.5	1.3

[†]Days to flower (DTF) in days, leaves 9-11 length (L) in cm, and area (A) in cm^2, and leaf (L), stem (STM), stalk (STK), and shoot (S) fresh (FW) and dry (DW) weight in g.

[‡]Standard error.

[§]*t*-test calculation of significance level at which parental allele means are different.

[¶]Diffference between the means of the high parent allele (RTx7000) and the low parent allele (BTx642).

[#]High parent allele (RTx7000) mean divided by the low parent allele (BTx642) mean.

CHAPTER X

CONCLUSION

The sorghum genotypes, BTx642 and RTx7000 were analyzed to identify traits that distinguish these genotypes at various stages of development when grown in well-watered conditions in the greenhouse and under field conditions. The two genotypes and RILs derived from them were found to differ in leaf size (length, width, and area), shoot biomass (stalk, stem, leaf, and leaf sheath weight), stalk and stem length, flowering time, root traits (length, surface area, and volume, number of tips and forks, and biomass) and panicle biomass accumulation. QTL mapping was utilized to characterize the genetic basis of trait variation and possible relationships among the traits analyzed and previously identified drought tolerance traits. The traits were measured at two developmental stages including the onset of GS3 (anthesis), and at the end of GS3 (grain maturity). In many instances, QTL affected several related traits (for example, different types of root traits) but QTL infrequently had an impact on traits associated with different organs. Several QTL were identified in only one environment. Some of this specificity of QTL detection by organ system was expected due to small population size that limited detection of QTL with small effects. However, the specificity observed is consistent with independent genetic control of traits associated with leaves, stems and roots. Overall, the genetic basis of variation in 72 traits was analyzed in the RIL population derived from BTx642 and RTx7000 and a total of 477 QTL were identified. Correlations between variation in root, leaf, and stalk traits and variation in shoot and panicle biomass were investigated. Nine of the QTL that modulated shoot biomass

accumulation acted independent of flowering time. Of these, four shoot biomass QTL co-localized with leaf size traits. Eight QTL for panicle biomass were detected with two coincident with QTL for upper leaf size. A QTL for leaf width at anthesis was found to co-localize with a stay green locus. These correlations provide the starting point for further analysis into the genetic basis of the traits analyzed and the correlations between leaf, stem, and root traits analyzed and the accumulation of biomass and grain in sorghum.

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APPENDIX A

Table A.1. BTx642 RIL population marker genetic and physical positions and segregation.

Marker	LG‡	Genetic Position§	Physical Position¶	Marker Segregation#	Marker	LG‡	Genetic Position§	Physical Position¶	Marker Segregation#
DG1	1	0.0	1,132,065	1.0:1.0	DG48	1	104.7	51,643,389	1.0:1.1
DG2	1	1.1	1,415,784	1.0:1.0	DG49	1	107.1	51,826,648	1.0:1.3
DG3	1	1.7	1,824,907	1.0:1.1	DG50	1	108.3	52,217,159	1.0:1.2
DG4	1	2.8	1,964,168	1.0:1.1	DG51	1	108.9	52,647,802	1.0:1.3
DG5	1	3.9	2,077,689	1.0:1.1	DG52	1	109.5	52,825,706	1.0:1.2
DG6	1	6.1	2,578,518	1.0:1.0	DG53	1	113.2	53,516,081	1.0:1.4
DG7	1	6.8	3,335,768	1.0:1.0	DG54	1	114.3	53,679,180	1.0:1.6*
DG8	1	7.4	3,425,227	1.1:1.0	DG55	1	114.9	53,775,483	1.0:1.5
DG9	1	8.5	3,752,972	1.2:1.0	DG56	1	116.0	54,216,748	1.0:1.5
DG10	1	10.9	5,520,295	1.2:1.0	DG57	1	116.6	54,509,305	1.0:1.4
DG11	1	11.2	5,886,097	1.3:1.0	DG58	1	117.3	54,650,601	1.0:1.4
DG12	1	11.5	5,886,400	1.2:1.0	DG59	1	123.8	55,657,892	1.0:1.3
DG13	1	12.4	5,975,406	1.2:1.0	DG60	1	125.6	55,779,723	1.0:1.3
DG14	1	13.3	6,457,030	1.2:1.0	DG61	1	128.0	56,245,572	1.0:1.1
DG15	1	13.9	6,978,152	1.3:1.0	DG62	1	131.0	56,707,521	1.0:1.0
DG16	1	14.6	7,315,100	1.3:1.0	DG63	1	132.1	57,141,926	1.0:1.1
DG17	1	25.6	11,843,676	1.4:1.0	DG64	1	133.9	57,158,862	1.0:1.1
DG18	1	26.7	12,045,722	1.4:1.0	DG65	1	142.3	58,108,968	1.0:1.0
DG19	1	30.4	13,179,511	1.3:1.0	DG66	1	142.9	59,563,845	1.0:1.0
DG20	1	31.5	13,183,391	1.3:1.0	DG67	1	144.0	60,210,749	1.0:1.0
DG21	1	32.1	13,300,793	1.2:1.0	DG68	1	145.8	60,469,973	1.0:1.2
DG22	1	38.4	13,616,615	1.4:1.0	DG69	1	146.4	60,510,211	1.0:1.1
DG23	1	65.7	16,465,677	1.0:1.0	DG70	1	147.5	60,802,865	1.0:1.1
DG24	1	67.1	17,661,610	1.0:1.0	DG71	1	154.6	64,482,728	1.0:1.0
DG25	1	69.1	18,272,587	1.0:1.1	DG72	1	155.8	64,836,055	1.0:1.0
DG26	1	69.8	18,290,873	1.0:1.1	DG73	1	156.4	65,124,032	1.0:1.0
DG27	1	72.8	19,149,886	1.0:1.0	DG74	1	157.1	65,202,064	1.0:1.0
DG28	1	78.7	21,904,980	1.3:1.0	DG75	1	160.1	65,441,121	1.0:1.1
DG29	1	80.5	23,893,666	1.3:1.0	DG76	1	160.8	65,752,402	1.0:1.0
DG30	1	84.2	25,959,826	1.1:1.0	DG77	1	162.0	65,905,618	1.1:1.0
DG31	1	85.4	28,297,645	1.1:1.0	DG78	1	162.6	65,987,823	1.1:1.0
DG32	1	86.0	43,997,458	1.2:1.0	DG79	1	163.2	67,166,985	1.1:1.0
DG33	1	89.6	46,114,704	1.0:1.0	DG80	1	165.7	67,379,971	1.3:1.0
DG34	1	90.3	46,978,614	1.0:1.0	DG81	1	167.9	67,785,160	1.2:1.0
DG35	1	90.9	47,071,208	1.0:1.0	DG82	1	182.0	71,099,630	1.3:1.0
DG36	1	91.5	47,241,118	1.0:1.0	DG83	1	183.2	71,191,866	1.2:1.0
DG37	1	92.4	47,401,666	1.0:1.0	DG84	1	185.0	71,837,886	1.3:1.0
DG38	1	93.3	48,632,804	1.0:1.0	DG85	1	186.2	72,089,436	1.1:1.0
DG39	1	93.9	48,731,498	1.1:1.0	DG86	1	186.8	72,107,669	1.2:1.0
DG40	1	95.1	49,676,750	1.0:1.0	DG87	1	188.0	72,288,774	1.2:1.0
DG41	1	95.7	50,404,250	1.0:1.1	DG88	1	188.6	72,435,660	1.1:1.0
DG42	1	96.3	50,779,967	1.0:1.1	DG89	1	190.4	72,938,507	1.0:1.0
DG43	1	98.7	50,859,432	1.0:1.1	DG90	2	0.0	443,839	1.1:1.0
DG44	1	99.9	50,944,662	1.0:1.1	DG91	2	2.8	1,588,403	1.0:1.0
DG45	1	102.3	51,073,170	1.0:1.1	DG92	2	4.1	1,816,469	1.0:1.0
DG46	1	103.5	51,522,284	1.0:1.1	DG93	2	5.9	2,353,353	1.0:1.0
DG47	1	104.1	51,603,230	1.0:1.0	DG94	2	7.7	2,637,749	1.1:1.0

Table A.1, continued.

Marker	LG[†]	Genetic Position[‡]	Physical Position[§]	Marker Segregation[¶]	Marker	LG[†]	Genetic Position[‡]	Physical Position[§]	Marker Segregation[¶]
DG95	2	8.8	2,935,224	1.3:1.0	DG145	3	0.0	421,680	1.0:1.4
DG96	2	9.9	3,413,744	1.1:1.0	DG146	3	3.7	1,177,165	1.0:1.2
DG97	2	10.5	3,687,906	1.2:1.0	DG147	3	4.3	1,447,138	1.0:1.1
DG98	2	13.5	3,967,168	1.0:1.0	DG148	3	4.9	1,452,327	1.0:1.2
DG99	2	14.6	4,219,271	1.1:1.0	DG149	3	6.0	1,638,809	1.0:1.1
DG100	2	15.2	4,432,241	1.2:1.0	DG150	3	7.3	1,896,975	1.0:1.1
DG101	2	16.5	4,720,368	1.2:1.0	DG151	3	8.6	1,967,752	1.0:1.0
DG102	2	22.9	4,899,014	1.3:1.0	DG152	3	9.2	1,991,940	1.0:1.1
DG103	2	27.0	5,470,637	1.3:1.0	DG153	3	11.2	2,087,154	1.0:1.2
DG104	2	30.1	5,600,328	1.1:1.0	DG154	3	13.0	2,235,920	1.0:1.1
DG105	2	33.2	6,411,540	1.3:1.0	DG155	3	13.6	2,391,356	1.0:1.1
DG106	2	34.4	6,512,381	1.3:1.0	DG156	3	14.2	2,495,314	1.0:1.2
DG107	2	39.2	6,873,304	1.3:1.0	DG157	3	14.9	2,959,214	1.0:1.2
DG108	2	41.4	7,866,848	1.2:1.0	DG158	3	16.8	3,146,576	1.0:1.2
DG109	2	43.3	8,404,730	1.2:1.0	DG159	3	18.9	3,439,252	1.0:1.1
DG110	2	62.7	13,927,457	1.3:1.0	DG160	3	19.5	4,103,048	1.0:1.0
DG111	2	63.9	47,369,715	1.4:1.0	DG161	3	20.1	4,376,702	1.0:1.1
DG112	2	64.5	52,332,890	1.3:1.0	DG162	3	22.5	4,761,641	1.0:1.1
DG113	2	65.1	53,502,998	1.3:1.0	DG163	3	23.6	5,735,108	1.0:1.2
DG114	2	67.1	55,085,091	1.3:1.0	DG164	3	26.9	6,187,728	1.0:1.3
DG115	2	68.3	55,332,398	1.3:1.0	DG165	3	28.1	6,407,283	1.0:1.4
DG116	2	69.5	55,580,245	1.3:1.0	DG166	3	32.1	7,119,901	1.0:1.4
DG117	2	70.9	56,695,852	1.5:1.0	DG167	3	33.2	7,328,503	1.0:1.4
DG118	2	72.7	56,956,264	1.6:1.0*	DG168	3	35.0	7,543,264	1.0:1.4
DG119	2	74.8	57,560,924	1.6:1.0*	DG169	3	35.6	7,940,280	1.0:1.4
DG120	2	76.2	58,017,037	1.7:1.0*	DG170	3	38.0	8,150,254	1.0:1.6*
DG121	2	80.4	58,867,163	1.8:1.0**	DG171	3	39.1	8,348,932	1.0:1.7*
DG122	2	81.7	59,242,855	1.8:1.0**	DG172	3	39.7	8,469,387	1.0:1.6*
DG123	2	82.3	59,579,955	1.7:1.0*	DG173	3	40.3	8,485,419	1.0:1.7*
DG124	2	84.7	59,949,245	1.6:1.0*	DG174	3	40.9	8,584,948	1.0:1.6*
DG125	2	89.1	60,696,862	1.5:1.0	DG175	3	41.5	8,723,202	1.0:1.6*
DG126	2	89.7	61,374,549	1.4:1.0	DG176	3	44.5	9,985,015	1.0:1.6*
DG127	2	91.0	61,543,010	1.4:1.0	DG177	3	46.4	10,102,706	1.0:1.5
DG128	2	92.3	61,759,693	1.4:1.0	DG178	3	48.3	10,391,393	1.0:1.6*
DG129	2	94.3	62,441,950	1.3:1.0	DG179	3	51.0	10,683,496	1.0:1.6*
DG130	2	98.3	63,514,945	1.3:1.0	DG180	3	52.8	11,160,047	1.0:1.6*
DG131	2	103.9	65,244,625	1.1:1.0	DG181	3	53.9	12,523,497	1.0:1.6*
DG132	2	105.0	65,731,619	1.1:1.0	DG182	3	55.3	12,756,524	1.0:1.8**
DG133	2	106.8	68,327,380	1.1:1.0	DG183	3	56.7	12,951,283	1.0:1.7*
DG134	2	110.5	69,615,903	1.0:1.2	DG184	3	57.8	13,438,186	1.0:1.7*
DG135	2	111.1	69,669,691	1.0:1.2	DG185	3	59.9	13,800,535	1.0:1.6*
DG136	2	112.0	69,695,964	1.0:1.2	DG186	3	61.3	15,468,062	1.0:1.8**
DG137	2	112.9	69,841,630	1.0:1.2	DG187	3	64.2	16,366,752	1.0:1.7*
DG138	2	113.5	69,910,974	1.0:1.3	DG188	3	64.9	45,545,540	1.0:1.6*
DG139	2	116.5	70,665,664	1.0:1.6*	DG189	3	66.0	51,454,564	1.0:1.6*
DG140	2	117.1	70,816,798	1.0:1.5	DG190	3	69.2	53,322,260	1.0:1.6*
DG141	2	120.1	71,422,224	1.0:1.4	DG191	3	72.4	54,415,446	1.0:1.5
DG142	2	120.7	71,789,496	1.0:1.3	DG192	3	74.3	54,934,613	1.0:1.4
DG143	2	121.3	72,330,181	1.0:1.4	DG193	3	75.0	55,441,525	1.0:1.4
DG144	2	125.6	73,082,593	1.0:1.5	DG194	3	75.9	55,892,236	1.0:1.4

Table A.1, continued.

Marker	LG[†]	Genetic Position[‡]	Physical Position[§]	Marker Segregation[¶]	Marker	LG[†]	Genetic Position[‡]	Physical Position[§]	Marker Segregation[¶]
DG195	3	77.4	56,238,895	1.0:1.4	DG245	4	8.4	1,504,225	1.0:1.5
DG196	3	78.2	56,782,235	1.0:1.3	DG246	4	34.5	6,252,057	1.3:1.0
DG197	3	81.3	57,226,675	1.0:1.2	DG247	4	35.6	6,882,201	1.3:1.0
DG198	3	82.6	57,333,004	1.0:1.2	DG248	4	37.4	7,229,813	1.2:1.0
DG199	3	84.4	57,803,734	1.0:1.0	DG249	4	38.0	7,662,188	1.3:1.0
DG200	3	85.5	57,928,680	1.0:1.0	DG250	4	39.2	7,943,180	1.4:1.0
DG201	3	87.9	58,555,059	1.0:1.1	DG251	4	41.6	8,675,620	1.4:1.0
DG202	3	89.1	58,969,358	1.0:1.1	DG252	4	42.8	8,823,351	1.3:1.0
DG203	3	90.3	59,366,053	1.0:1.1	DG253	4	44.6	8,996,627	1.2:1.0
DG204	3	91.5	59,560,463	1.0:1.1	DG254	4	48.3	9,429,083	1.0:1.1
DG205	3	92.1	59,753,639	1.0:1.1	DG255	4	49.9	10,094,021	1.0:1.0
DG206	3	93.9	60,110,046	1.0:1.1	DG256	4	50.7	10,266,058	1.0:1.0
DG207	3	94.5	60,214,313	1.0:1.1	DG257	4	53.5	10,372,107	1.1:1.0
DG208	3	95.1	60,677,970	1.0:1.0	DG258	4	54.8	11,349,743	1.0:1.0
DG209	3	95.7	60,815,578	1.0:1.0	DG259	4	57.8	12,453,144	1.0:1.1
DG210	3	96.5	61,392,844	1.0:1.0	DG260	4	58.4	12,769,338	1.0:1.1
DG211	3	98.1	61,551,692	1.1:1.0	DG261	4	59.0	44,309,611	1.0:1.1
DG212	3	98.7	61,863,809	1.0:1.0	DG262	4	60.1	48,920,288	1.0:1.2
DG213	3	99.3	62,085,512	1.1:1.0	DG263	4	61.2	49,146,918	1.0:1.1
DG214	3	100.0	63,522,585	1.1:1.0	DG264	4	62.3	50,416,388	1.0:1.1
DG215	3	102.7	64,376,683	1.1:1.0	DG265	4	66.0	50,903,774	1.0:1.2
DG216	3	104.1	64,646,936	1.1:1.0	DG266	4	66.8	51,039,067	1.0:1.1
DG217	3	104.7	65,525,393	1.1:1.0	DG267	4	72.2	52,271,490	1.0:1.1
DG218	3	105.6	66,195,293	1.1:1.0	DG268	4	73.4	52,527,732	1.0:1.2
DG219	3	106.5	66,434,503	1.0:1.0	DG269	4	74.0	53,085,906	1.0:1.3
DG220	3	107.6	66,752,140	1.0:1.1	DG270	4	75.1	53,321,429	1.0:1.3
DG221	3	109.4	67,307,937	1.0:1.0	DG271	4	75.7	53,544,604	1.0:1.2
DG222	3	112.4	67,939,827	1.0:1.0	DG272	4	76.8	53,785,100	1.0:1.2
DG223	3	113.0	68,104,297	1.0:1.0	DG273	4	77.9	54,213,289	1.0:1.2
DG224	3	114.1	68,982,966	1.1:1.0	DG274	4	79.0	54,685,548	1.0:1.1
DG225	3	114.7	69,116,135	1.2:1.0	DG275	4	80.8	55,047,686	1.0:1.0
DG226	3	115.9	69,180,887	1.2:1.0	DG276	4	81.4	55,663,213	1.0:1.0
DG227	3	119.0	69,728,620	1.2:1.0	DG277	4	83.2	56,242,356	1.0:1.2
DG228	3	125.8	70,230,977	1.5:1.0	DG278	4	87.6	56,502,255	1.0:1.0
DG229	3	127.2	70,597,525	1.7:1.0*	DG279	4	93.5	58,861,819	1.0:1.4
DG230	3	132.0	71,832,221	1.8:1.0**	DG280	4	94.6	58,864,301	1.0:1.4
DG231	3	135.4	72,173,211	1.6:1.0*	DG281	4	95.2	58,933,629	1.0:1.4
DG232	3	136.0	72,281,810	1.4:1.0	DG282	4	95.8	58,935,877	1.0:1.3
DG233	3	137.2	72,711,436	1.3:1.0	DG283	4	98.2	59,064,346	1.0:1.3
DG234	3	137.8	73,145,525	1.4:1.0	DG284	4	98.8	59,160,896	1.0:1.4
DG235	3	140.2	73,658,620	1.5:1.0*	DG285	4	100.6	59,838,225	1.0:1.3
DG236	4	0.0	409,173	1.0:1.6*	DG286	4	101.2	60,153,138	1.0:1.2
DG237	4	3.0	779,254	1.0:1.4	DG287	4	101.8	60,313,060	1.0:1.1
DG238	4	3.6	822,462	1.0:1.5	DG288	4	103.0	61,030,000	1.0:1.3
DG239	4	4.2	963,252	1.0:1.5	DG289	4	103.6	61,070,326	1.0:1.2
DG240	4	4.8	1,008,603	1.0:1.5*	DG290	4	104.2	61,609,908	1.0:1.1
DG241	4	5.4	1,072,471	1.0:1.5*	DG291	4	105.9	61,673,294	1.0:1.1
DG242	4	6.0	1,180,449	1.0:1.5	DG292	4	106.5	61,883,554	1.0:1.2
DG243	4	6.6	1,253,605	1.0:1.6*	DG293	4	107.1	61,968,138	1.0:1.1
DG244	4	7.2	1,357,179	1.0:1.5	DG294	4	107.7	62,073,072	1.0:1.1

Table A.1, continued.

Marker	LG[†]	Genetic Position[‡]	Physical Position[§]	Marker Segregation[¶]
DG295	4	108.3	62,160,158	1.0:1.0
DG296	4	109.0	62,237,760	1.0:1.1
DG297	4	111.1	62,327,962	1.0:1.2
DG298	4	111.8	62,339,651	1.0:1.2
DG299	4	113.0	62,670,633	1.0:1.2
DG300	4	121.4	63,335,696	1.0:1.1
DG301	4	122.1	63,754,521	1.0:1.1
DG302	4	124.3	64,061,219	1.0:1.1
DG303	4	124.9	64,268,215	1.0:1.1
DG304	4	126.7	64,400,937	1.0:1.1
DG305	4	128.5	64,611,864	1.0:1.2
DG306	4	129.1	64,794,148	1.0:1.2
DG307	4	130.3	65,021,164	1.0:1.2
DG308	4	131.5	65,463,145	1.0:1.3
DG309	4	133.9	65,667,820	1.0:1.1
DG310	4	137.0	66,392,573	1.0:1.1
DG311	4	138.5	66,646,029	1.0:1.1
DG312	4	139.2	66,691,746	1.0:1.2
DG313	4	140.6	66,981,782	1.0:1.2
DG314	4	142.0	67,116,462	1.0:1.3
DG315	4	143.8	67,496,079	1.0:1.3
DG316	5	0.0	1,001,370	1.2:1.0
DG317	5	2.4	1,515,435	1.1:1.0
DG318	5	6.7	2,160,363	1.4:1.0
DG319	5	8.0	2,631,902	1.4:1.0
DG320	5	8.7	2,679,478	1.5:1.0
DG321	5	13.8	3,048,406	1.2:1.0
DG322	5	19.5	3,620,140	1.6:1.0*
DG323	5	36.3	5,097,351	1.3:1.0
DG324	5	37.5	5,325,838	1.2:1.0
DG325	5	41.9	6,523,469	1.0:1.0
DG326	5	43.1	6,857,282	1.0:1.1
DG327	5	47.4	7,352,477	1.0:1.0
DG328	5	48.0	7,512,402	1.1:1.0
DG329	5	48.6	8,166,610	1.1:1.0
DG330	5	49.2	8,856,612	1.1:1.0
DG331	5	52.9	11,125,059	1.0:1.2
DG332	5	55.9	11,952,173	1.0:1.3
DG333	5	57.1	12,504,766	1.0:1.3
DG334	5	58.9	16,063,396	1.0:1.4
DG335	5	59.5	45,245,338	1.0:1.3
DG336	5	60.1	45,735,280	1.0:1.3
DG337	5	60.7	46,317,685	1.0:1.3
DG338	5	63.1	48,602,588	1.0:1.4
DG339	5	67.5	50,009,642	1.0:1.1
DG340	5	68.1	50,039,469	1.0:1.2
DG341	5	68.7	50,901,522	1.0:1.3
DG342	5	69.3	51,124,239	1.0:1.2
DG343	5	71.7	51,517,151	1.0:1.1
DG344	5	72.3	51,908,606	1.0:1.1
DG345	5	78.8	53,179,234	1.0:1.0
DG346	5	83.3	53,972,424	1.0:1.0
DG347	5	84.0	54,592,959	1.0:1.1
DG348	5	88.7	55,308,844	1.0:1.2
DG349	5	90.1	55,598,516	1.0:1.2
DG350	5	91.9	56,083,187	1.0:1.3
DG351	5	92.5	56,607,774	1.0:1.3
DG352	5	94.3	57,152,585	1.0:1.2
DG353	5	94.9	57,638,122	1.0:1.1
DG354	5	95.5	57,892,430	1.0:1.2
DG355	5	97.5	58,166,144	1.0:1.1
DG356	5	100.6	58,600,158	1.1:1.0
DG357	5	103.3	58,848,641	1.1:1.0
DG358	5	104.9	59,036,023	1.1:1.0
DG359	5	105.7	59,163,352	1.1:1.0
DG360	5	106.8	59,791,753	1.1:1.0
DG361	5	107.9	59,979,787	1.1:1.0
DG362	5	109.7	60,289,946	1.1:1.0
DG363	5	110.3	60,347,791	1.1:1.0
DG364	5	111.4	60,737,286	1.1:1.0
DG365	5	112.5	60,819,851	1.1:1.0
DG366	5	114.3	62,159,020	1.3:1.0
DG367	6	0.0	334,114	1.0:1.3
DG368	6	3.5	950,408	1.0:1.2
DG369	6	5.5	1,032,887	1.0:1.1
DG370	6	26.5	3,449,360	1.3:1.0
DG371	6	29.1	4,108,756	1.2:1.0
DG372	6	29.7	4,711,341	1.1:1.0
DG373	6	33.8	6,428,223	1.1:1.0
DG374	6	34.9	32,354,931	1.1:1.0
DG375	6	36.2	40,120,199	1.0:1.0
DG376	6	36.9	40,201,125	1.0:1.0
DG377	6	39.0	41,856,240	1.0:1.1
DG378	6	41.5	42,728,446	1.0:1.1
DG379	6	43.1	42,795,561	1.0:1.0
DG380	6	46.8	44,708,620	1.1:1.0
DG381	6	58.0	45,730,642	1.2:1.0
DG382	6	59.2	45,819,571	1.2:1.0
DG383	6	60.1	46,247,880	1.2:1.0
DG384	6	61.0	46,394,567	1.3:1.0
DG385	6	61.8	46,688,291	1.4:1.0
DG386	6	63.5	46,942,206	1.4:1.0
DG387	6	66.1	47,513,584	1.2:1.0
DG388	6	66.7	47,513,904	1.1:1.0
DG389	6	69.7	48,297,388	1.2:1.0
DG390	6	70.3	48,499,624	1.1:1.0
DG391	6	71.4	48,740,934	1.1:1.0
DG392	6	72.0	49,176,678	1.1:1.0
DG393	6	72.6	49,772,604	1.0:1.0
DG394	6	74.4	50,347,055	1.0:1.1

Table A.1, continued.

Marker	LG[†]	Genetic Position[‡]	Physical Position[§]	Marker Segregation[¶]
DG395	6	75.0	51,139,358	1.0:1.1
DG396	6	75.6	51,499,148	1.0:1.1
DG397	6	78.0	52,094,273	1.0:1.0
DG398	6	79.8	52,338,107	1.0:1.0
DG399	6	82.8	53,328,895	1.0:1.1
DG400	6	84.0	53,555,398	1.0:1.0
DG401	6	85.7	53,664,189	1.0:1.0
DG402	6	86.5	53,731,684	1.0:1.0
DG403	6	87.1	53,830,249	1.0:1.1
DG404	6	88.3	54,030,226	1.0:1.2
DG405	6	90.7	54,574,665	1.0:1.4
DG406	6	91.4	54,678,188	1.0:1.3
DG407	6	92.0	54,722,172	1.0:1.3
DG408	6	92.7	54,793,840	1.0:1.3
DG409	6	93.4	54,874,250	1.0:1.3
DG410	6	95.5	55,095,011	1.0:1.2
DG411	6	96.2	55,157,298	1.0:1.3
DG412	6	97.3	55,318,076	1.0:1.3
DG413	6	98.4	55,997,638	1.0:1.1
DG414	6	99.0	56,230,045	1.0:1.0
DG415	6	99.6	56,303,525	1.0:1.0
DG416	6	101.4	56,393,047	1.0:1.0
DG417	6	103.2	56,586,356	1.0:1.0
DG418	6	105.6	57,222,080	1.0:1.1
DG419	6	107.3	57,729,673	1.0:1.2
DG420	6	109.0	58,326,028	1.0:1.2
DG421	6	109.7	58,461,757	1.0:1.1
DG422	6	110.3	58,558,664	1.0:1.0
DG423	6	110.9	58,810,612	1.0:1.0
DG424	6	119.3	60,847,137	1.1:1.0
DG425	6	121.1	61,702,168	1.1:1.0
DG426	7	0.0	749,128	1.0:1.1
DG427	7	1.1	1,108,402	1.0:1.1
DG428	7	1.7	1,259,767	1.0:1.0
DG429	7	4.7	1,592,131	1.0:1.0
DG430	7	5.3	1,593,847	1.0:1.1
DG431	7	7.1	1,744,990	1.0:1.0
DG432	7	8.2	1,906,209	1.0:1.0
DG433	7	15.7	2,416,258	1.1:1.0
DG434	7	16.4	2,530,604	1.1:1.0
DG435	7	17.7	2,617,617	1.1:1.0
DG436	7	19.8	2,806,192	1.2:1.0
DG437	7	21.0	2,956,486	1.0:1.0
DG438	7	23.4	3,285,205	1.0:1.0
DG439	7	25.9	3,456,139	1.2:1.0
DG440	7	27.5	3,765,149	1.1:1.0
DG441	7	28.1	3,861,739	1.1:1.0
DG442	7	28.7	3,952,824	1.1:1.0
DG443	7	29.8	4,204,880	1.0:1.0
DG444	7	30.4	4,252,105	1.0:1.0
DG445	7	37.9	5,101,119	1.0:1.1
DG446	7	39.1	5,180,259	1.0:1.0
DG447	7	40.9	5,186,838	1.0:1.0
DG448	7	43.3	5,910,106	1.0:1.0
DG449	7	46.9	6,610,312	1.0:1.2
DG450	7	47.6	6,702,491	1.0:1.3
DG451	7	50.0	6,807,644	1.0:1.3
DG452	7	51.8	7,115,441	1.0:1.3
DG453	7	52.4	8,240,709	1.0:1.2
DG454	7	53.5	9,253,224	1.0:1.2
DG455	7	54.1	9,662,976	1.0:1.2
DG456	7	55.2	52,727,156	1.0:1.1
DG457	8	0.0	679,896	1.4:1.0
DG458	8	9.2	2,133,644	1.4:1.0
DG459	8	10.4	2,449,617	1.4:1.0
DG460	8	11.0	2,476,297	1.4:1.0
DG461	8	18.6	3,300,851	1.4:1.0
DG462	8	19.9	3,339,660	1.3:1.0
DG463	8	28.1	5,330,131	1.4:1.0
DG464	8	36.3	6,254,008	1.5:1.0
DG465	8	43.0	41,623,973	1.5:1.0
DG466	8	48.5	47,392,453	1.5:1.0
DG467	8	50.2	47,538,857	1.4:1.0
DG468	8	52.3	48,141,665	1.3:1.0
DG469	8	54.1	48,788,347	1.3:1.0
DG470	8	54.7	48,946,305	1.4:1.0
DG471	8	55.8	49,032,189	1.4:1.0
DG472	8	56.9	49,587,492	1.3:1.0
DG473	8	57.7	49,740,362	1.3:1.0
DG474	8	61.6	50,116,222	1.2:1.0
DG475	8	65.9	50,817,616	1.0:1.0
DG476	8	71.1	51,148,148	1.1:1.0
DG477	8	72.4	51,513,364	1.1:1.0
DG478	8	73.0	51,620,632	1.0:1.0
DG479	8	73.6	51,707,009	1.0:1.0
DG480	8	74.9	52,075,617	1.0:1.0
DG481	8	75.5	52,443,992	1.1:1.0
DG482	8	80.4	53,057,572	1.0:1.0
DG483	8	81.0	53,493,827	1.0:1.0
DG484	8	82.6	53,662,358	1.0:1.1
DG485	8	83.4	53,679,232	1.0:1.0
DG486	8	84.0	54,322,182	1.0:1.0
DG487	8	85.1	54,362,584	1.1:1.0
DG488	9	0.0	99,298	1.2:1.0
DG489	9	1.8	1,066,712	1.1:1.0
DG490	9	3.0	1,113,094	1.0:1.0
DG491	9	6.7	1,321,837	1.0:1.1
DG492	9	7.3	1,569,213	1.0:1.1
DG493	9	7.9	1,590,989	1.0:1.2
DG494	9	9.7	1,712,415	1.0:1.1

Table A.1, continued.

Marker	LG[†]	Genetic Position[‡]	Physical Position[§]	Marker Segregation[¶]	Marker	LG[†]	Genetic Position[‡]	Physical Position[§]	Marker Segregation[¶]
DG495	9	11.5	1,990,168	1.0:1.1	DG545	10	63.7	48,084,225	2.3:1.0***
DG496	9	13.3	2,250,695	1.0:1.1	DG546	10	64.3	49,342,394	2.5:1.0***
DG497	9	16.5	2,421,341	1.2:1.0	DG547	10	66.8	49,835,384	2.3:1.0***
DG498	9	17.7	2,452,699	1.1:1.0	DG548	10	68.0	50,088,782	2.0:1.0***
DG499	9	24.9	2,774,240	1.4:1.0	DG549	10	68.6	50,913,625	1.9:1.0**
DG500	9	26.7	2,970,840	1.3:1.0	DG550	10	69.2	51,120,566	1.8:1.0**
DG501	9	28.4	3,221,188	1.4:1.0	DG551	10	76.2	52,397,690	1.3:1.0
DG502	9	29.7	3,554,346	1.3:1.0	DG552	10	76.9	52,525,422	1.3:1.0
DG503	9	31.0	4,001,262	1.3:1.0	DG553	10	77.5	52,773,863	1.2:1.0
DG504	9	33.0	4,281,712	1.1:1.0	DG554	10	78.6	53,022,379	1.1:1.0
DG505	9	34.2	4,578,792	1.1:1.0	DG555	10	80.7	53,232,079	1.2:1.0
DG506	9	34.8	5,102,229	1.1:1.0	DG556	10	82.1	53,551,604	1.1:1.0
DG507	9	35.9	5,461,749	1.0:1.0	DG557	10	83.9	53,832,398	1.0:1.0
DG508	10	0.0	91,868	1.3:1.0	DG558	10	85.0	54,280,442	1.0:1.0
DG509	10	1.1	597,201	1.3:1.0	DG559	10	86.8	54,784,154	1.1:1.0
DG510	10	2.2	1,068,310	1.4:1.0	DG560	10	92.7	55,282,386	1.0:1.0
DG511	10	4.4	1,122,881	1.2:1.0	DG561	10	94.5	55,524,582	1.0:1.1
DG512	10	5.1	1,158,646	1.3:1.0	DG562	10	99.9	56,396,650	1.0:1.1
DG513	10	6.9	1,182,389	1.4:1.0	DG563	10	101.6	56,511,327	1.0:1.1
DG514	10	7.5	1,271,772	1.3:1.0	DG564	10	104.6	56,728,379	1.0:1.0
DG515	10	8.1	1,601,728	1.4:1.0	DG565	10	108.2	56,922,188	1.0:1.0
DG516	10	9.4	1,756,183	1.5:1.0*	DG566	10	118.8	59,063,056	1.0:1.3
DG517	10	12.8	1,870,626	1.7:1.0*					
DG518	10	13.5	1,901,431	1.6:1.0*					
DG519	10	15.3	2,238,012	1.5:1.0					
DG520	10	21.7	2,856,016	1.5:1.0					
DG521	10	23.1	3,117,217	1.5:1.0					
DG522	10	24.2	3,530,346	1.6:1.0*					
DG523	10	24.8	3,807,169	1.7:1.0*					
DG524	10	25.9	4,236,688	1.6:1.0*					
DG525	10	27.0	4,500,466	1.6:1.0*					
DG526	10	27.6	4,690,915	1.6:1.0*					
DG527	10	28.2	4,766,568	1.6:1.0*					
DG528	10	31.1	5,515,900	1.6:1.0*					
DG529	10	32.1	5,570,527	1.5:1.0					
DG530	10	35.7	5,965,824	1.5:1.0*					
DG531	10	36.3	6,047,494	1.6:1.0*					
DG532	10	40.3	6,439,000	1.7:1.0*					
DG533	10	42.7	6,881,556	1.7:1.0*					
DG534	10	43.3	7,119,121	1.8:1.0**					
DG535	10	44.1	7,503,740	1.8:1.0**					
DG536	10	45.7	7,711,232	2.0:1.0**					
DG537	10	46.8	8,056,948	2.0:1.0**					
DG538	10	47.9	8,483,361	2.0:1.0**					
DG539	10	49.0	8,592,781	2.2:1.0***					
DG540	10	49.6	8,876,673	2.3:1.0***					
DG541	10	56.3	10,075,847	2.9:1.0***					
DG542	10	61.4	12,145,020	2.6:1.0***					
DG543	10	62.5	14,568,592	2.3:1.0***					
DG544	10	63.1	45,853,718	2.5:1.0***					

[†]Linkage group.

[‡]Centimorgan.

[§]Base pairs.

[¶]Ratio of RTx7000 to BTx642 alleles for a given marker. Significant segregation distortion is denoted by: (*) P<0.05, (**) P<0.01, (***) P<0.001.

APPENDIX B

Table B.1. BTx642 RIL population QTL for plants grown in greenhouse conditions and field conditions in 2008-2010.

Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
Days to Flower	1	2008	80.5	3.92	80.1 - 84.2	79.9 - 85.5	-1.2	0.10	3.07
			90.9	4.60	86.9 - 94.1	85.4 - 95.4	-1.4	0.12	3.07
		2009	78.7	4.85	74.1 - 82.2	70.5 - 85.5	-1.5	0.11	3.17
		GH	14.3	6.44	13.5 - 20.4	12.6 - 22.8	2.2	0.11	3.14
	3	2009	33.3	4.92	29.6 - 35.2	24.2 - 37.4	1.5	0.11	3.17
	6	2008	34.9	6.31	33.2 - 37.2	31.3 - 38.2	1.8	0.16	3.07
		2009	34.8	6.31	30.9 - 36.0	26.8 - 36.5	2.0	0.15	3.17
			47.0	4.10	46.6 - 53.5	46.4 - 55.8	1.7	0.09	3.17
		2010	26.5	8.19	26.2 - 28.5	26.1 - 29.1	2.5	0.22	3.05
			36.9	12.17	35.3 - 38.1	34.6 - 38.4	3.1	0.30	3.05
	8	2008	46.1	3.25	42.9 - 49.3	42.7 - 50.2	-1.3	0.11	3.07
			55.8	5.90	55.1 - 56.9	54.4 - 58.8	-1.5	0.15	3.07
		2009	55.9	6.59	55.0 - 57.1	52.5 - 57.8	-1.8	0.16	3.17
			64.4	5.29	61.6 - 65.8	61.6 - 65.8	-1.7	0.14	3.17
		2010	46.5	4.92	42.9 - 50.3	42.9 - 50.3	-1.8	0.13	3.05
			56.9	7.09	55.1 - 61.2	51.8 - 64.1	-2.0	0.16	3.05
		GH	59.8	5.98	57.0 - 65.6	54.3 - 65.8	-2.1	0.10	3.14
	10	2008	59.4	4.70	53.4 - 62.6	50.5 - 65.7	-1.8	0.13	3.07
		2009	59.4	8.53	57.2 - 60.8	56.1 - 61.5	-3.1	0.23	3.17
		2010	58.8	6.14	53.9 - 61.3	51.8 - 61.9	-2.6	0.15	3.05
		GH	58.3	9.20	52.1 - 61.6	51.1 - 62.4	-3.5	0.19	3.14
Stalk Length	6	2009	41.5	25.06	40.6 - 42.7	40.1 - 42.8	11.6	0.60	3.10
		2010	40.9	19.49	39.6 - 42.7	39.1 - 43.0	13.1	0.43	3.22
			70.5	4.47	68.7 - 71.3	66.9 - 72.8	4.2	0.06	3.22
		GH	42.3	19.92	40.1 - 44.0	39.7 - 45.0	8.5	0.49	3.27
			89.1	4.75	86.5 - 91.1	84.2 - 98.4	3.3	0.08	3.27
	8	2010	66.0	3.54	65.8 - 71.8	65.7 - 85.0	-3.9	0.04	3.22
			77.5	3.63	74.1 - 80.5	65.7 - 85.0	-4.2	0.05	3.22
Shoot Biomass	SFW	1	2009	131.0	4.28	128.8 - 133.3	128.2 - 137.0	-25.01	0.09	3.17
	SDW			131.0	3.65	128.8 - 133.5	125.0 - 142.2	-5.34	0.09	3.10
	STKFW			131.1	3.52	128.3 - 133.9	117.1 - 139.1	-17.57	0.07	2.98
	LDW		2010	69.3	3.40	67.3 - 72.3	67.1 - 73.2	-1.41	0.06	3.01
	SDW	2	2009	31.8	6.08	29.6 - 34.2	26.5 - 35.4	7.29	0.17	3.10
	LDW	3	2009	24.1	3.18	20.8 - 28.1	18.9 - 34.6	1.98	0.10	3.19
	LFW			35.1	3.67	32.1 - 37.8	23.8 - 45.7	8.40	0.11	3.12

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
	STMFW	3	GH	98.1	3.75	96.2 - 108.0	92.0 - 108.9	-1.32	0.11	3.19
	SFW	4	2010	3.1	3.17	0.3 - 6.3	0.0 - 7.4	20.46	0.05	2.98
	LSDW			3.1	3.61	0.0 - 4.8	0.0 - 8.4	0.95	0.05	2.87
	LFW			53.7	3.36	43.5 - 56.0	42.6 - 58.0	6.09	0.07	3.04
	LDW		GH	54.7	4.62	51.5 - 56.3	49.9 - 57.7	0.43	0.09	2.99
	SDW			54.7	3.85	50.5 - 56.6	49.0 - 57.5	0.88	0.06	3.02
	LFW			54.8	4.88	52.0 - 56.4	50.3 - 57.2	2.14	0.09	2.94
	STKDW	5	2009	97.6	3.63	95.0 - 103.5	84.0 - 104.9	4.17	0.09	3.03
	SFW		2010	99.6	4.40	95.2 - 103.6	90.0 - 106.2	25.74	0.08	2.98
	LFW			100.8	4.41	97.7 - 103.8	95.4 - 106.6	6.88	0.09	3.04
	SDW			102.4	4.45	95.9 - 106.1	94.8 - 107.4	5.35	0.09	3.05
	LDW			102.6	4.30	98.2 - 106.5	95.1 - 109.8	1.60	0.09	3.01
	LSDW			103.2	4.38	98.3 - 105.6	95.4 - 107.0	1.13	0.08	2.87
Shoot Biomass	STKDW	6	2009	29.2	7.47	27.4 - 29.7	26.8 - 34.4	6.15	0.21	3.03
	SFW			32.7	6.90	30.7 - 34.9	29.7 - 36.1	34.33	0.16	3.17
	STKFW			33.8	9.16	30.9 - 34.4	30.0 - 35.1	31.60	0.22	2.98
	SFW			40.1	5.36	36.3 - 42.2	26.7 - 44.2	32.30	0.14	3.17
	LFW			89.9	3.23	86.6 - 92.2	82.4 - 101.0	7.73	0.10	3.12
	SFW			93.4	4.89	88.8 - 95.2	79.7 - 96.9	27.72	0.11	3.17
	STMDW		2010	29.0	7.84	26.5 - 29.4	14.6 - 37.7	3.26	0.17	2.82
	STMDW			34.8	10.20	34.3 - 35.9	28.4 - 36.4	4.54	0.21	2.82
	LFW			34.9	9.68	34.2 - 36.3	29.5 - 37.3	13.31	0.23	3.04
	SFW			34.9	12.49	34.4 - 36.2	33.9 - 38.5	56.20	0.29	2.98
	STKFW			34.9	10.66	34.2 - 36.1	30.5 - 38.1	42.67	0.23	2.78
	STKDW			34.9	14.39	34.6 - 35.7	34.2 - 36.1	9.72	0.32	2.86
	LSFW			34.9	13.11	34.4 - 36.0	34.0 - 36.9	14.07	0.39	2.79
	LSDW			34.9	13.32	34.5 - 36.2	34.1 - 36.8	2.69	0.29	2.87
	STMFW			35.0	10.71	33.9 - 36.5	28.2 - 38.5	22.56	0.19	2.91
	SDW			35.0	10.61	34.3 - 36.1	33.8 - 37.5	10.47	0.26	3.05
	LDW			35.1	9.12	34.3 - 37.2	33.7 - 38.2	2.85	0.21	3.01
	STMFW			74.4	4.73	72.3 - 75.2	66.6 - 76.8	13.74	0.08	2.91
	STMDW		GH	42.1	3.51	37.6 - 45.0	33.8 - 46.5	0.21	0.09	3.19
	STMFW			90.7	8.50	88.8 - 94.5	87.9 - 95.5	2.10	0.29	3.19
	STKFW	8	2009	55.8	5.07	54.6 - 57.8	45.9 - 65.7	-22.03	0.10	2.98
	LFW			56.9	4.74	55.2 - 57.8	54.2 - 71.0	-9.69	0.14	3.12
	LDW			56.9	6.39	55.5 - 61.0	55.0 - 66.9	-2.84	0.20	3.19
	STKDW			57.0	4.73	54.6 - 60.9	44.0 - 66.3	-4.90	0.12	3.03
	SFW			67.0	4.57	61.6 - 70.5	55.8 - 79.7	-27.97	0.11	3.17

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
Shoot Biomass	LFW	8	2010	55.8	8.73	55.3 - 62.1	55.0 - 64.6	-10.89	0.20	3.04
	SDW			55.8	7.21	55.2 - 57.8	54.9 - 57.8	-8.03	0.18	3.05
	LSDW			55.8	5.13	55.4 - 57.9	55.0 - 65.0	-1.50	0.12	2.87
	LDW			55.9	8.04	55.4 - 57.8	55.0 - 63.6	-2.63	0.22	3.01
	STMDW			55.9	6.53	55.3 - 57.6	55.0 - 64.0	-3.41	0.19	2.82
	STMFW			56.9	6.79	55.7 - 61.7	55.0 - 66.0	-16.62	0.12	2.91
	STKDW			56.9	6.31	55.3 - 63.6	52.6 - 65.0	-4.94	0.12	2.86
	STMDW			56.9	6.19	55.2 - 57.8	53.0 - 64.4	-3.27	0.11	2.82
	SFW			61.3	7.10	55.5 - 64.2	55.0 - 65.8	-37.69	0.18	2.98
	SDW			61.6	7.01	57.8 - 64.2	57.8 - 65.4	-8.38	0.20	3.05
	STKFW			61.6	7.17	55.6 - 66.0	55.4 - 72.2	-27.35	0.15	2.78
	STMFW			62.7	6.42	61.4 - 66.0	61.1 - 72.2	-18.68	0.15	2.95
	STMFW			68.9	5.94	65.9 - 71.0	65.9 - 71.0	-16.14	0.12	2.91
	LSFW			78.3	4.48	73.3 - 81.9	61.3 - 83.4	-6.53	0.11	2.79
	LSFW		GH	28.2	3.57	23.3 - 36.3	18.8 - 36.3	-1.48	0.07	2.95
	LSFW			38.7	3.36	36.3 - 42.5	36.3 - 42.5	-1.59	0.08	2.95
	LSDW			39.5	3.43	29.4 - 47.1	28.0 - 48.5	-0.24	0.06	2.98
	LDW			52.2	7.06	50.4 - 54.4	44.0 - 63.2	-0.53	0.12	2.99
	SFW			52.2	5.43	49.0 - 54.0	48.6 - 54.8	-6.15	0.10	2.99
	LFW			52.3	6.39	50.0 - 53.8	44.1 - 54.8	-2.20	0.11	2.94
	STKDW			56.9	4.74	51.5 - 61.6	50.2 - 63.8	-0.48	0.09	3.01
	STMDW			58.8	3.99	55.2 - 63.1	50.3 - 64.8	-0.21	0.11	3.19
	SDW			58.8	6.37	52.0 - 62.4	50.4 - 64.3	-1.22	0.12	3.02
	SFW			59.0	5.08	54.8 - 63.3	54.8 - 65.0	-5.88	0.10	2.99
	STKFW	10	2010	54.7	4.57	52.1 - 56.3	50.7 - 60.6	-32.20	0.13	2.78
	LSFW			54.7	5.62	51.1 - 56.1	49.9 - 60.7	-10.58	0.18	2.79
	LFW			55.6	8.46	53.5 - 59.2	52.2 - 60.3	-15.39	0.23	3.04
	SFW			56.1	5.26	51.5 - 58.6	50.0 - 60.4	-39.73	0.11	2.98
	SDW			56.2	5.08	51.3 - 59.2	50.0 - 61.0	-8.16	0.11	3.05
	LSDW			56.2	5.69	51.5 - 60.0	50.1 - 61.3	-1.92	0.11	2.87
	LDW			56.3	5.82	52.2 - 60.5	50.5 - 61.8	-2.63	0.13	3.01
	STMDW		GH	54.9	6.32	51.8 - 58.8	50.6 - 60.3	-0.33	0.21	3.19
	STKDW			55.5	10.16	52.3 - 59.4	51.2 - 60.5	-0.90	0.24	3.01
	LSFW			55.6	10.88	53.1 - 59.3	52.0 - 60.2	-4.07	0.32	2.95
	STKFW			56.1	9.18	52.3 - 59.4	51.2 - 60.4	-5.42	0.25	3.12
	LFW			56.3	19.36	53.8 - 58.7	52.5 - 59.6	-6.00	0.51	2.94
	LDW			56.3	19.78	54.6 - 58.1	53.3 - 59.6	-1.18	0.48	2.99
	SFW			56.3	16.99	54.8 - 57.8	53.2 - 59.4	-14.32	0.46	2.99

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
	SDW			56.3	13.74	54.3 - 57.9	53.2 - 59.1	-2.55	0.34	3.02
	LSDW	10	GH	57.4	14.58	53.5 - 59.7	52.4 - 60.6	-0.64	0.35	2.98
Panicle Biomass	PNDW	1	2009	77.8	3.77	73.7 - 80.5	67.3 - 84.6	5.42	0.15	3.08
	PNFW			7.7	7.33	6.4 - 8.7	5.2 - 11.7	-8.65	0.21	3.24
	PNDW		2008	7.7	6.42	6.3 - 9.2	4.9 - 11.9	-7.79	0.19	3.02
	PNFW			71.0	4.04	69.2 - 72.5	65.7 - 74.6	4.42	0.13	3.00
	PNDW	2	2010	71.0	4.56	69.3 - 72.5	65.2 - 76.2	3.82	0.15	3.08
	PNFW			69.4	4.39	68.9 - 73.8	60.5 - 77.2	6.07	0.11	3.24
	PNDW		2008	69.4	3.72	66.8 - 75.4	60.3 - 78.4	5.50	0.10	3.02
	PNDW	5	2010	89.7	3.57	87.1 - 92.4	84.5 - 100.1	3.41	0.12	3.08
Leaf Size	L14A	1		91.6	4.54	88.0 - 93.9	86.1 - 95.3	-17.9	0.13	3.03
	L14L		2008	91.6	3.84	86.9 - 93.6	84.8 - 94.4	-2.5	0.11	2.98
	L13A			93.4	3.28	90.9 - 97.9	87.4 - 103.5	-18.5	0.09	3.12
	L14A			15.7	5.44	14.2 - 21.0	11.6 - 23.7	15.7	0.17	3.12
	L13A			16.6	9.81	14.5 - 21.5	13.9 - 23.6	31.2	0.26	3.15
	L12L			17.0	5.23	13.9 - 22.5	8.2 - 24.8	2.3	0.15	3.27
	L12A			17.7	5.41	14.5 - 23.7	14.3 - 26.0	24.6	0.15	3.19
	L13L		2009	21.8	4.49	15.8 - 26.5	14.5 - 28.8	2.4	0.14	3.13
	L13W			22.1	6.31	15.4 - 25.0	14.5 - 25.1	0.44	0.22	3.22
	L14W			28.9	3.64	21.0 - 35.0	14.6 - 37.6	0.27	0.11	3.12
	L12W			31.4	3.25	27.1 - 40.2	15.1 - 49.3	0.23	0.08	3.16
	L12W			128.6	4.21	125.1 - 131.8	121.1 - 133.8	-0.29	0.11	3.16
	L10W			6.1	5.27	3.5 - 7.9	2.3 - 13.2	0.53	0.13	3.02
	L11W			52.1	3.20	41.1 - 62.6	38.2 - 79.1	-0.56	0.21	3.05
	L11W			69.3	3.53	67.1 - 72.3	38.2 - 79.1	-0.31	0.11	3.05
	L14A			97.4	7.75	94.5 - 98.6	93.0 - 100.1	-20.5	0.20	3.10
	L14W		2010	97.6	3.96	95.7 - 102.7	94.2 - 105.9	-0.31	0.11	3.14
	L13L			100.3	4.12	96.6 - 102.9	92.5 - 104.6	-2.1	0.11	3.04
	L13A			100.9	8.83	98.0 - 102.1	96.8 - 103.2	-26.4	0.20	3.14
	L14L			113.2	6.59	111.0 - 114.6	109.6 - 115.5	-2.5	0.17	3.18
	L12W			113.4	5.62	111.6 - 115.4	110.5 - 116.4	-0.45	0.15	3.25
	L14W			1.2	3.42	0.0 - 2.9	0.0 - 11.0	0.20	0.06	3.05
	L11W			2.9	6.35	1.8 - 5.3	0.0 - 6.5	0.20	0.10	3.16
	L9W			3.8	5.84	2.6 - 6.2	0.0 - 7.0	0.30	0.08	3.10
	L11A		GH	4.0	3.83	2.2 - 6.0	0.0 - 7.0	10.2	0.05	3.12
	L12W			4.0	3.23	2.9 - 6.7	1.8 - 7.0	0.20	0.05	3.18
	TLA			4.0	3.78	0.0 - 5.8	0.0 - 8.1	61.8	0.06	3.15
	L9A			4.1	4.03	2.4 - 6.7	0.0 - 13.4	10.7	0.06	3.03

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
	L10W			5.0	5.24	0.6 - 6.7	0.0 - 12.6	0.30	0.09	3.10
	L9W			9.4	5.10	6.7 - 11.4	7.0 - 13.4	0.30	0.07	3.10
	L10A			11.0	4.87	8.8 - 14.5	6.8 - 21.5	12.7	0.07	3.04
	L12A	1	GH	29.2	3.26	21.2 - 34.4	15.8 - 36.2	9.8	0.04	2.99
	L11A			32.2	3.45	19.4 - 34.6	15.0 - 36.4	9.6	0.04	3.12
	L14A			91.3	3.86	90.5 - 93.5	81.7 - 95.7	-11.5	0.08	2.97
	L10A			114.5	3.33	113.1 - 117.5	104.0 - 125.4	-9.5	0.05	3.04
	L9L			125.6	3.64	119.3 - 127.4	116.7 - 131.7	-2.5	0.06	3.13
	L9W			2.8	3.86	0.0 - 5.6	0.0 - 13.2	-0.30	0.11	3.03
	L10W			3.8	3.98	0.0 - 7.8	0.0 - 13.1	-0.29	0.12	3.04
	L9W			10.6	3.73	8.3 - 12.9	0.0 - 13.2	-0.30	0.10	3.03
	L12W			55.0	3.47	44.7 - 66.0	42.9 - 68.4	0.29	0.14	3.09
	L14W			64.5	3.94	63.3 - 68.3	62.7 - 70.7	0.25	0.11	3.14
	L13L			106.8	3.94	103.9 - 110.1	93.9 - 116.3	2.2	0.11	3.00
	L13W			112.9	6.57	111.7 - 119.5	110.8 - 120.4	0.35	0.19	3.15
	L11W			112.9	4.46	109.3 - 120.9	107.3 - 126.0	0.29	0.14	3.15
	L13A		2008	117.2	4.40	113.7 - 120.6	106.5 - 123.9	22.4	0.12	3.12
	L12A			119.2	3.84	115.0 - 126.0	106.7 - 126.0	18.8	0.11	3.01
Leaf Size	L12W			119.2	8.30	114.3 - 120.6	112.5 - 122.2	0.40	0.26	3.09
	L11A			120.1	3.57	117.1 - 126.0	108.3 - 126.0	17.6	0.11	3.04
	L12L			120.9	4.53	118.0 - 123.9	116.6 - 126.0	1.8	0.12	3.07
	L14W			121.2	5.39	117.6 - 126.0	114.4 - 126.0	0.31	0.16	3.14
	TLA	2		123.8	4.17	117.4 - 126.0	111.0 - 126.0	143.2	0.13	3.03
	L10W			126.0	5.56	122.8 - 126.0	120.9 - 126.0	0.34	0.16	3.04
	L9L			14.7	3.39	10.4 - 21.3	7.7 - 23.0	-2.5	0.09	3.16
	L14A			94.4	5.91	92.9 - 96.5	91.6 - 97.7	14.3	0.16	3.12
	L13A			94.4	6.40	92.7 - 97.0	90.7 - 98.3	21.9	0.14	3.15
	L13L			95.4	3.75	90.7 - 102.9	89.5 - 105.0	2.1	0.10	3.13
	L12W		2009	113.3	9.96	112.3 - 116.2	111.8 - 118.3	0.46	0.29	3.16
	L12A			113.7	4.48	111.6 - 116.3	110.6 - 120.9	20.8	0.12	3.19
	L11W			113.7	3.81	111.0 - 116.7	106.9 - 120.6	0.27	0.12	3.14
	L11A			115.5	4.11	111.4 - 120.7	107.3 - 126.0	18.3	0.10	3.06
	TLA			116.6	3.61	111.4 - 120.0	106.0 - 120.9	111.7	0.09	2.98
	L10A			8.9	3.96	7.8 - 12.5	6.0 - 15.3	-26.1	0.07	3.14
	L14W			116.6	4.43	115.2 - 118.0	114.2 - 119.8	0.34	0.11	3.14
	L13A		2010	116.6	5.39	114.0 - 117.3	112.8 - 121.4	20.8	0.11	3.14
	L12A			116.6	3.93	113.7 - 124.8	106.8 - 126.0	20.5	0.10	3.10
	L14A			116.7	3.36	114.5 - 122.5	113.6 - 126.0	12.5	0.07	3.10

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
	L12W		2010	117.2	5.06	115.2 - 119.8	114.0 - 121.3	0.41	0.13	3.25
	L11W			8.8	5.07	0.0 - 12.5	0.0 - 14.2	-0.20	0.08	3.16
	TLA			8.8	4.93	7.8 - 11.3	2.9 - 13.0	-70.1	0.07	3.15
	L12A	2	GH	9.0	5.02	6.9 - 11.6	2.9 - 13.0	-13.6	0.08	2.99
	L11A			9.0	6.00	7.1 - 12.0	3.2 - 13.2	-14.8	0.09	3.12
	L9A			9.9	3.13	8.3 - 12.8	5.9 - 14.8	-8.4	0.04	3.03
	L12L			10.5	4.25	8.2 - 13.4	7.1 - 20.0	-1.9	0.08	3.18
	L12A		2008	40.9	4.19	38.4 - 41.5	34.1 - 44.3	20.3	0.12	3.01
	L10A			23.6	5.23	22.4 - 26.6	22.4 - 35.6	25.0	0.07	3.11
	L9A			23.8	4.48	21.0 - 26.3	19.8 - 28.1	30.8	0.12	3.22
	TLA			24.1	4.06	22.4 - 28.1	22.3 - 38.2	119.4	0.10	2.98
	L11A			33.2	3.43	22.4 - 35.5	22.3 - 44.0	16.4	0.08	3.06
	L10A		2009	33.3	4.72	28.4 - 35.2	22.4 - 35.6	25.0	0.07	3.11
	L9A			33.3	3.64	28.6 - 33.4	12.1 - 33.4	28.9	0.10	3.22
	L9W			33.3	3.81	22.4 - 35.3	22.2 - 37.7	0.41	0.12	3.09
	L10W	3		33.4	3.62	22.4 - 36.5	22.4 - 44.0	0.32	0.11	3.25
	L10W			40.3	3.36	38.3 - 41.7	38.3 - 41.7	0.30	0.10	3.25
	TLA			40.5	3.36	38.1 - 43.7	22.2 - 44.2	112.3	0.09	2.98
Leaf Size	L14L			104.8	4.70	101.6 - 109.3	99.2 - 112.5	-2.2	0.13	3.10
	L11W			114.1	4.91	111.3 - 115.7	110.1 - 118.1	-0.20	0.08	3.16
	L12A		GH	114.2	4.64	110.2 - 117.0	108.6 - 118.3	-12.8	0.07	2.99
	L11A			114.2	5.65	111.5 - 116.4	110.2 - 118.3	-14.1	0.08	3.12
	TLA			114.2	3.16	113.0 - 116.6	102.4 - 118.5	-52.7	0.05	3.15
	L14L			114.6	3.48	112.6 - 115.8	112.6 - 115.8	-1.9	0.10	3.10
	L11W		2008	53.6	3.15	45.6 - 56.1	40.8 - 58.2	0.23	0.08	3.15
	L14A			144.0	3.63	140.8 - 144.0	138.5 - 144.0	17.0	0.10	3.03
	L10W			52.8	5.40	49.8 - 54.6	46.9 - 57.0	0.53	0.14	3.02
	L9A			53.6	4.35	46.8 - 55.1	43.8 - 57.5	31.2	0.08	3.14
	L13A			124.4	10.92	123.1 - 125.3	122.9 - 126.2	34.6	0.25	3.14
	L13W			124.4	5.14	123.2 - 125.4	122.5 - 126.3	0.40	0.16	3.09
	L12W	4		124.4	4.12	122.9 - 126.3	117.1 - 127.9	0.37	0.10	3.25
	L13L		2010	124.7	4.21	122.5 - 126.8	121.3 - 128.1	2.2	0.11	3.04
	L14W			124.9	6.92	122.5 - 126.1	121.4 - 126.7	0.53	0.20	3.14
	L12A			125.1	6.46	123.7 - 126.4	122.9 - 126.9	30.7	0.16	3.10
	L14A			131.6	4.90	131.5 - 137.0	131.5 - 137.0	17.2	0.14	3.10
	L14W			131.6	3.68	130.6 - 137.0	129.2 - 137.0	0.36	0.11	3.14
	L14L			141.6	8.02	139.9 - 144.0	139.1 - 144.0	2.7	0.21	3.18
	TLA		GH	50.8	3.21	49.1 - 55.5	45.2 - 57.0	48.8	0.04	3.15

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
Leaf Size	L12A	4	GH	51.0	4.21	49.8 - 54.3	48.4 - 56.3	10.5	0.05	2.99
	L12W			53.6	5.54	51.5 - 56.4	50.0 - 57.4	0.20	0.09	3.18
	L10A			54.7	6.01	52.6 - 56.6	51.0 - 57.4	13.0	0.09	3.04
	L9A			54.8	5.66	50.8 - 56.7	49.9 - 57.7	11.4	0.08	3.03
	L10W			54.8	4.24	53.2 - 56.9	51.6 - 57.7	0.20	0.07	3.10
	L9W			54.8	5.81	52.6 - 57.0	50.6 - 57.7	0.20	0.08	3.10
	L12L	5	2008	94.4	4.88	92.7 - 102.1	92.0 - 103.4	1.9	0.14	3.07
	L10A			95.7	4.06	93.4 - 99.9	92.2 - 103.3	21.9	0.12	3.07
	L9L			95.7	5.05	93.4 - 100.5	92.5 - 103.1	2.7	0.14	3.13
	L11A			97.2	5.97	92.6 - 99.4	92.0 - 100.5	23.6	0.19	3.04
	L9A			98.6	5.05	95.4 - 103.1	93.7 - 104.9	26.7	0.14	3.08
	L11L			98.7	3.13	93.5 - 104.4	91.9 - 108.0	1.4	0.07	3.09
	TLA			99.6	4.26	93.2 - 102.3	92.1 - 103.3	136.5	0.13	3.03
	L12A			100.7	3.29	97.9 - 103.3	90.1 - 106.4	16.9	0.09	3.01
	L10L			103.4	4.31	95.4 - 105.1	93.7 - 106.8	2.0	0.10	3.12
	L9W		2009	89.7	3.96	83.4 - 92.1	80.1 - 95.0	0.45	0.13	3.09
	L9A			97.6	3.48	95.2 - 104.4	89.2 - 104.8	27.4	0.09	3.22
	L11A			97.7	7.35	95.9 - 100.0	95.0 - 101.0	24.6	0.19	3.06
	TLA			97.7	5.57	95.2 - 101.7	92.5 - 104.3	142.0	0.15	2.98
	L10A			97.9	6.29	95.3 - 100.5	91.9 - 103.3	29.5	0.18	3.11
	L11L			98.5	4.25	95.6 - 103.2	92.8 - 106.9	1.7	0.10	3.13
	L10L			99.0	3.68	95.6 - 104.8	93.3 - 108.5	2.1	0.11	3.17
	L11W		2010	57.2	3.41	53.4 - 58.7	49.3 - 67.0	-0.37	0.09	3.05
	L12A			94.4	6.27	92.6 - 97.1	91.9 - 103.2	25.4	0.16	3.10
	L12L			94.4	6.96	92.3 - 99.4	91.8 - 103.1	2.8	0.19	3.14
	L11A			98.7	4.73	95.5 - 104.4	93.5 - 108.0	24.1	0.10	3.06
	L9A			100.7	3.77	98.0 - 108.0	95.4 - 110.2	28.5	0.07	3.14
	L10W			100.8	3.25	98.7 - 103.8	95.4 - 105.0	0.38	0.08	3.02
	TLA			100.8	4.40	97.7 - 103.7	90.9 - 105.1	164.6	0.12	3.01
	L11L			104.3	7.18	99.3 - 107.0	96.8 - 109.9	2.8	0.13	3.27
	L10L			105.7	7.04	101.2 - 107.0	99.6 - 109.2	3.6	0.13	3.18
	L9L			105.8	4.13	99.5 - 109.6	98.4 - 110.1	3.2	0.07	3.09
	L10A			106.7	4.78	100.6 - 109.7	97.9 - 110.3	27.8	0.08	3.14
	L9L	6	2008	8.3	7.05	3.7 - 16.1	0.2 - 19.6	3.8	0.27	3.13
	L10A			11.9	3.55	0.0 - 21.8	0.0 - 25.8	24.0	0.15	3.07
	L12L			13.0	3.33	4.4 - 25.8	0.0 - 26.2	1.8	0.12	3.07
	L9A			13.8	5.50	5.3 - 22.9	5.0 - 25.8	35.2	0.24	3.08
	TLA			31.1	5.32	27.9 - 36.3	26.4 - 39.4	151.2	0.13	3.03

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
Leaf Size	L11L	6	2008	34.9	13.10	33.8 - 37.8	32.1 - 41.2	3.6	0.39	3.09
	L10L			34.9	12.52	34.0 - 36.2	31.3 - 36.3	4.5	0.37	3.12
	L14W			66.1	3.81	63.7 - 66.6	61.6 - 68.9	-0.26	0.12	3.14
	L10W		2009	29.1	4.76	26.9 - 31.8	18.0 - 33.2	0.41	0.14	3.25
	TLA			29.7	5.78	28.2 - 32.8	26.4 - 33.9	144.6	0.17	2.98
	L11A			30.6	9.03	29.0 - 33.3	27.1 - 34.0	29.5	0.25	3.06
	L10A			30.9	8.04	29.1 - 33.3	27.1 - 35.1	38.0	0.25	3.11
	L9A			32.7	4.47	28.9 - 35.9	26.8 - 38.9	36.5	0.14	3.22
	L9L			35.9	5.62	33.9 - 38.0	30.2 - 42.9	3.9	0.17	3.16
	L11L			36.1	12.45	34.5 - 37.9	30.7 - 38.6	3.3	0.34	3.13
	L14L			36.3	5.85	30.1 - 36.6	26.2 - 36.6	3.1	0.22	3.11
	L13L			37.0	10.61	34.8 - 38.2	34.2 - 38.8	3.8	0.33	3.13
	L12L			37.0	12.75	36.3 - 38.7	35.5 - 40.0	3.7	0.37	3.27
	L10L			37.9	7.81	34.6 - 41.6	30.7 - 43.0	3.7	0.25	3.17
	L14L			70.4	3.34	68.6 - 72.3	64.3 - 79.7	-2.2	0.10	3.11
	L12L		2010	8.7	5.42	2.6 - 19.9	0.6 - 24.5	2.7	0.18	3.14
	TLA			12.6	5.15	6.2 - 21.3	3.7 - 25.0	213.6	0.18	3.01
	L9W			14.2	5.94	4.4 - 22.7	0.0 - 25.7	0.76	0.25	3.23
	L10W			30.9	6.54	29.2 - 33.9	13.8 - 37.8	0.62	0.18	3.02
	L11L			34.9	17.48	34.6 - 35.7	34.3 - 36.3	6.5	0.43	3.27
	L9A			34.9	13.70	34.3 - 35.9	29.7 - 36.5	73.2	0.34	3.14
	L9L			34.9	16.28	34.4 - 36.6	33.9 - 37.8	8.9	0.39	3.09
	L11A			35.0	13.57	34.6 - 37.4	34.4 - 38.2	58.7	0.36	3.06
	L10L			35.0	16.90	34.4 - 37.4	34.0 - 38.1	8.4	0.42	3.18
	L10A			37.0	18.48	35.8 - 37.9	34.7 - 38.3	80.3	0.45	3.14
	L10W	8	2008	2.4	3.56	0.0 - 19.5	0.0 - 28.1	-0.25	0.11	3.04
	L11W			10.3	5.06	3.9 - 19.8	0.4 - 26.9	-0.33	0.15	3.15
	L11A			10.4	4.31	0.3 - 19.4	0.0 - 24.8	-20.0	0.13	3.04
	L9W			10.4	4.63	2.2 - 18.2	0.0 - 28.0	-0.35	0.13	3.03
	L10A			10.5	3.81	0.0 - 21.6	0.0 - 28.1	-21.2	0.11	3.07
	L9A			16.7	6.01	11.2 - 22.9	0.0 - 27.8	-31.6	0.19	3.08
	L9L			18.6	5.98	13.8 - 21.2	11.1 - 26.1	-3.1	0.17	3.13
	L10L			55.9	4.07	55.0 - 60.3	52.5 - 65.6	-2.0	0.09	3.12
	L14A			56.9	3.65	55.9 - 59.3	53.8 - 61.6	-16.4	0.10	3.03
	L14L			56.9	5.32	56.3 - 59.8	55.7 - 61.3	-2.9	0.16	2.98
	L13A			56.9	3.81	55.2 - 60.5	52.4 - 65.2	-20.2	0.10	3.12
	L12A			56.9	4.64	55.4 - 60.5	54.8 - 65.4	-20.4	0.13	3.01
	TLA			56.9	4.07	55.7 - 60.8	54.9 - 65.6	-135.5	0.12	3.03

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
	L13L		2008	57.8	3.25	55.9 - 61.3	55.4 - 65.4	-2.0	0.10	3.00
	L9L		2009	55.8	4.10	52.8 - 57.9	44.9 - 71.2	-2.8	0.12	3.16
	L9W			57.0	3.33	54.2 - 59.3	45.3 - 71.2	-0.38	0.10	3.09
	L9A			67.0	4.78	55.8 - 70.2	55.7 - 79.9	-34.8	0.15	3.22
	L9L			69.1	4.05	62.4 - 71.2	62.4 - 71.2	-3.0	0.13	3.16
	L14W		2010	18.6	4.36	10.0 - 22.5	4.7 - 27.1	-0.35	0.12	3.14
	L9W			18.6	6.35	13.6 - 25.1	11.2 - 28.0	-0.72	0.20	3.23
	L13W			31.1	3.17	12.1 - 36.1	0.9 - 36.1	-0.32	0.10	3.09
	L14L			36.2	3.33	31.4 - 42.1	28.7 - 43.1	-1.8	0.09	3.18
	L14A			38.4	3.71	32.2 - 42.5	28.5 - 48.2	-14.2	0.09	3.10
	L13L			50.3	3.22	39.3 - 59.8	36.3 - 61.1	-1.9	0.08	3.04
	L10W			55.8	7.32	55.3 - 57.9	55.0 - 61.7	-0.63	0.20	3.02
	L11L			55.9	4.63	54.7 - 57.9	52.5 - 65.7	-2.4	0.08	3.27
	L9L			56.9	9.09	55.2 - 57.6	54.8 - 64.1	-5.3	0.18	3.09
	TLA			56.9	5.09	55.4 - 61.8	55.0 - 65.3	-183.7	0.13	3.01
	L9A	8		57.0	8.95	55.3 - 58.0	55.0 - 65.6	-48.5	0.18	3.14
	L10L			62.7	6.04	55.1 - 65.5	52.9 - 71.1	-3.7	0.12	3.18
	L11A			73.1	4.50	69.0 - 74.8	61.1 - 83.0	-24.6	0.09	3.06
Leaf Size	L10A			73.1	6.95	71.5 - 73.9	67.1 - 75.0	-35.8	0.13	3.14
	L9A		GH	41.4	6.88	37.7 - 47.7	35.8 - 48.6	-14.2	0.11	3.03
	L9W			42.0	6.75	38.2 - 47.8	36.9 - 48.6	-0.30	0.10	3.10
	L9L			44.9	5.90	42.9 - 48.1	42.9 - 48.1	-3.7	0.13	3.13
	L9A			52.3	6.88	50.4 - 53.8	48.6 - 53.5	-13.4	0.10	3.03
	L9W			53.3	6.41	50.3 - 53.5	50.3 - 53.5	-0.30	0.09	3.10
	L10W			55.8	5.35	51.4 - 59.7	50.3 - 61.6	-0.20	0.09	3.10
	L10A			57.8	4.93	56.6 - 61.0	49.9 - 66.7	-11.6	0.07	3.04
	L11L			57.8	5.14	55.2 - 60.4	53.1 - 62.4	-2.5	0.10	3.25
	L10L			57.8	6.07	55.8 - 60.7	52.3 - 61.6	-3.0	0.12	3.14
	L12W			57.9	3.92	55.9 - 62.9	49.7 - 65.2	-0.20	0.06	3.18
	L12A			74.9	3.65	72.5 - 79.4	69.2 - 82.8	-11.6	0.06	2.99
	L11A			75.0	3.36	72.5 - 79.0	67.3 - 82.7	-10.7	0.05	3.12
	L9W	10	2008	53.3	4.66	49.4 - 60.3	45.7 - 62.5	-0.40	0.14	3.03
	L10W			56.4	5.59	51.9 - 59.9	49.8 - 61.8	-0.40	0.17	3.04
	L9A			56.8	4.60	52.1 - 61.2	50.2 - 62.3	-33.5	0.14	3.08
	L10L			57.5	4.85	52.6 - 60.9	50.8 - 61.8	-3.3	0.13	3.12
	L11L			58.6	5.67	52.5 - 61.5	50.4 - 62.5	-2.8	0.15	3.09
	L12L			80.7	5.09	78.4 - 82.1	77.7 - 83.2	-2.0	0.14	3.07
	L14W		2009	42.7	3.75	39.2 - 47.2	35.1 - 48.2	-0.28	0.10	3.12

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
	L12W			46.8	6.95	46.1 - 48.0	44.4 - 49.5	-0.38	0.18	3.16
	L13W			49.1	7.13	48.3 - 55.0	46.5 - 61.3	-0.51	0.22	3.22
	L13A			56.4	7.91	54.4 - 59.1	52.6 - 60.2	-34.4	0.19	3.15
	L9A			56.4	5.88	53.6 - 60.9	51.4 - 62.1	-51.2	0.18	3.22
	L14A		2009	57.3	7.37	53.7 - 60.7	51.5 - 62.6	-20.3	0.22	3.12
	L9L			57.4	4.75	54.8 - 61.1	52.8 - 62.2	-4.4	0.16	3.16
	L10A			57.5	4.68	53.9 - 61.0	51.9 - 61.8	-35.3	0.14	3.11
	L12A			62.5	5.27	58.8 - 64.9	57.6 - 66.9	-31.1	0.14	3.19
	L14W			69.2	5.59	66.8 - 72.6	64.7 - 74.3	-0.36	0.17	3.12
	L12L			43.5	4.22	42.1 - 46.0	34.9 - 55.8	-2.3	0.11	3.14
	L14L			46.8	3.17	43.5 - 48.2	40.6 - 53.7	-1.8	0.07	3.18
	L9A			56.3	8.44	53.8 - 58.4	52.1 - 59.7	-62.5	0.17	3.14
	L9L			56.4	9.42	54.2 - 58.5	52.6 - 60.0	-7.4	0.19	3.09
	L10L		2010	58.3	9.09	54.3 - 61.4	51.9 - 62.7	-6.6	0.21	3.18
	L11L			59.3	12.80	56.9 - 61.2	56.1 - 61.4	-6.5	0.31	3.27
	L11A			61.2	10.08	58.8 - 62.6	57.4 - 63.0	-58.6	0.25	3.06
	L13W			61.3	3.19	58.4 - 62.6	56.8 - 66.5	-0.32	0.08	3.09
	L10A			62.4	9.40	59.2 - 62.8	57.5 - 63.2	-66.2	0.20	3.14
Leaf Size	L11W	10		52.5	7.83	49.7 - 56.2	49.7 - 56.2	-0.40	0.18	3.16
	L11A			54.6	13.11	50.1 - 56.1	49.6 - 56.1	-32.1	0.38	3.12
	L12A			54.7	12.80	51.9 - 56.0	50.2 - 56.1	-35.5	0.40	2.99
	L12L			55.5	8.69	52.7 - 55.8	51.0 - 56.0	-5.4	0.35	3.18
	L10A			56.3	14.86	54.3 - 58.1	53.2 - 59.2	-30.1	0.29	3.04
	L10L			56.3	15.73	54.4 - 57.8	53.1 - 60.4	-6.3	0.40	3.14
	L9L			56.3	15.76	53.6 - 58.6	51.7 - 60.1	-7.1	0.37	3.13
	L12W			56.3	8.76	54.8 - 58.4	53.4 - 59.7	-0.30	0.16	3.18
	L10W			56.3	14.36	54.9 - 57.9	53.6 - 59.1	-0.60	0.30	3.10
	L9W		GH	56.3	14.78	53.1 - 59.4	51.9 - 60.7	-0.60	0.26	3.10
	L9A			56.4	14.80	53.1 - 59.5	51.9 - 60.4	-29.4	0.30	3.03
	L11L			57.4	17.97	55.5 - 59.2	53.8 - 60.1	-6.4	0.53	3.25
	L13A			60.3	18.95	58.6 - 61.8	58.0 - 62.3	-45.8	0.59	3.06
	L14W			60.5	7.97	58.2 - 61.9	57.2 - 62.5	-0.40	0.20	3.05
	L14L			61.4	7.12	58.5 - 61.9	57.5 - 62.5	-3.6	0.26	3.10
	L13L			61.4	7.46	57.8 - 61.9	56.5 - 62.5	-3.6	0.25	3.11
	L12L			61.4	14.27	59.9 - 61.9	58.3 - 62.4	-6.4	0.38	3.18
	L13W			61.4	12.81	58.9 - 62.0	57.8 - 62.6	-0.50	0.31	2.98
	L11W			61.4	11.57	60.1 - 62.1	58.8 - 62.6	-0.40	0.21	3.16
	TLA			61.4	14.52	59.3 - 61.9	58.3 - 62.4	-176.6	0.31	3.15

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
Leaf	L14A	10	GH	61.5	11.97	58.6 - 62.1	57.6 - 62.6	-24.3	0.32	2.97
	L12A			61.5	10.88	59.2 - 62.1	58.1 - 62.6	-28.8	0.21	2.99
	L11A			61.5	11.11	59.6 - 62.1	58.3 - 62.7	-27.0	0.19	3.12
Root Size	RTIPS2	1	2009	132.0	3.20	128.9 - 139.7	125.7 - 142.5	-6.60	0.11	3.04
	RDW			132.3	4.30	129.4 - 133.9	127.4 - 142.3	-0.86	0.16	2.98
	RSA9			132.4	4.73	131.3 - 140.2	118.4 - 142.2	-2.60	0.18	3.12
	RV9			132.4	4.76	131.2 - 140.3	118.6 - 142.0	-0.28	0.19	3.12
	RL9			132.6	4.68	131.2 - 140.2	118.1 - 142.2	-1.97	0.18	3.12
	RL8			136.5	3.19	131.2 - 142.0	126.0 - 142.3	-2.19	0.14	3.08
	RV8			136.5	3.22	131.0 - 142.0	126.3 - 142.5	-0.24	0.13	3.07
	RSA8			136.7	3.18	131.3 - 142.0	126.3 - 142.3	-2.57	0.13	3.19
	RTIPS9			137.7	5.01	131.3 - 142.3	128.5 - 142.2	-0.28	0.19	3.06
	TRV			140.0	3.80	134.2 - 144.0	132.1 - 146.1	-1.36	0.16	3.29
	RL6			140.8	3.69	135.3 - 143.8	132.7 - 147.2	-4.23	0.16	3.08
	RSA6			140.8	3.73	135.5 - 144.0	132.6 - 146.9	-3.71	0.16	3.12
	RV6			140.9	3.75	135.5 - 143.8	132.7 - 147.2	-0.25	0.16	3.07
	RSA7			141.5	3.28	135.2 - 146.1	132.1 - 154.6	-2.97	0.13	3.12
	RV7			141.5	3.31	135.0 - 146.3	132.3 - 154.6	-0.23	0.23	3.07
	RL7			141.7	3.27	135.0 - 146.1	132.1 - 154.6	-2.92	0.12	3.15
	RTIPS6			143.1	4.16	137.7 - 144.1	135.0 - 146.6	-0.53	0.14	3.08
	RL8		2010	12.5	3.12	9.0 - 14.2	5.0 - 14.6	1.41	0.09	3.05
	RSA8			12.5	3.12	9.1 - 14.2	4.7 - 14.5	1.66	0.08	2.94
	RV8			12.5	3.16	9.1 - 14.2	5.0 - 14.5	0.15	0.09	2.96
	RL4			31.5	3.75	30.3 - 34.2	27.5 - 37.1	-4.28	0.12	3.12
	RSA4			31.5	3.76	30.1 - 34.1	27.7 - 37.0	-2.47	0.12	3.12
	RV4			31.5	3.74	30.3 - 34.1	27.7 - 37.1	-0.07	0.12	3.14
	RL5			31.7	3.09	27.4 - 35.6	23.0 - 44.0	-3.13	0.11	3.07
	RV2			77.3	4.28	73.1 - 81.1	71.1 - 83.1	0.04	0.14	3.07
	RSA2			77.5	4.53	73.2 - 80.7	71.1 - 82.8	2.22	0.15	3.17
	RL2			77.6	4.63	73.2 - 80.4	71.4 - 82.3	10.45	0.16	3.03
	RT8			145.8	5.74	143.2 - 146.8	137.1 - 155.8	-0.19	0.19	3.14
	RT5			146.0	4.55	136.2 - 147.6	132.1 - 153.6	-0.48	0.18	2.82
	RT2			154.6	3.50	150.5 - 156.0	147.5 - 159.8	-397	0.13	2.90
	RDW			154.6	6.31	151.8 - 156.2	150.0 - 157.2	-0.82	0.21	3.05
	RT4			155.9	3.13	151.9 - 159.0	148.4 - 160.7	-0.50	0.11	3.02
	RL3	2	2009	103.8	3.25	99.5 - 105.8	94.4 - 110.0	-4.93	0.12	3.13
	TRTIPS			103.9	3.25	100.2 - 106.2	95.6 - 110.5	-50	0.12	3.11
	RTIPS1			103.9	3.26	100.3 - 106.3	95.5 - 110.5	-32	0.12	3.10

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
	RL7			89.2	3.24	85.3 - 92.0	84.4 - 99.6	-2.77	0.11	3.15
	RSA7			89.3	3.27	85.3 - 92.2	84.3 - 99.7	-2.91	0.11	3.12
	RV7			89.3	3.29	85.2 - 92.0	92.1 - 97.8	-0.23	0.11	3.07
	TRV			94.6	3.61	92.8 - 97.1	85.1 - 99.6	-1.28	0.14	3.29
	RL5			95.1	3.48	93.0 - 97.9	84.9 - 99.9	-4.14	0.11	3.10
	RL6			95.1	3.37	92.7 - 97.5	85.1 - 99.9	-3.79	0.12	3.08
	RL7			95.1	3.24	92.3 - 97.2	84.4 - 99.6	-2.77	0.11	3.15
	RL8	3	2009	95.1	3.26	92.9 - 95.5	86.7 - 95.6	-2.72	0.12	3.08
	RSA5			95.1	3.50	92.9 - 97.7	84.9 - 99.9	-2.80	0.12	3.18
	RSA6			95.1	3.36	93.0 - 97.3	84.9 - 99.9	-3.16	0.12	3.12
	RSA7			95.1	3.27	92.5 - 97.4	84.3 - 99.7	-2.91	0.11	3.12
	RSA8			95.1	3.29	93.1 - 95.5	86.9 - 95.8	-3.03	0.11	3.19
	RV5			95.1	3.46	92.8 - 98.2	84.9 - 99.9	-0.16	0.11	3.32
	RV7			95.1	3.35	92.1 - 97.7	84.5 - 99.7	-0.24	0.11	3.07
	RV6			95.3	3.34	92.7 - 97.5	85.1 - 99.6	-0.23	0.12	3.07
	RV8			95.3	3.28	93.1 - 95.6	86.9 - 95.7	-0.30	0.12	3.07
Root Size	TRSA			39.3	3.12	37.1 - 41.2	32.3 - 49.5	14.42	0.11	3.10
	TRV			39.3	3.45	37.0 - 41.4	34.8 - 48.6	0.83	0.12	3.12
	RL5			39.3	3.13	38.0 - 41.5	36.1 - 49.1	3.70	0.10	3.12
	RSA5			39.3	3.15	38.2 - 41.4	36.2 - 49.5	2.04	0.10	3.12
	RV3			39.3	3.88	38.2 - 41.5	35.9 - 49.4	0.06	0.13	3.10
	RV5			39.3	3.14	38.0 - 41.4	36.2 - 49.5	0.09	0.10	3.14
	TRTIPS			53.6	3.31	51.7 - 56.2	48.7 - 57.7	410	0.12	2.73
	TFKS	4	2010	53.6	3.34	51.4 - 56.4	42.8 - 59.3	612	0.12	2.64
	RT7			53.6	4.87	51.5 - 54.9	50.1 - 56.9	0.28	0.18	3.07
	RL1			54.7	3.16	54.7 - 58.2	54.7 - 73.8	36.60	0.11	2.95
	RTIPS1			54.7	3.59	52.1 - 57.2	50.9 - 59.3	426	0.13	2.74
	RT3			54.9	3.53	54.7 - 57.0	51.7 - 58.3	4.13	0.15	2.90
	RL2			66.1	3.20	62.3 - 69.3	54.7 - 73.8	36.60	0.11	2.95
	RT6			72.2	4.01	64.7 - 74.0	63.2 - 75.4	0.30	0.14	3.07
	RT9			98.4	4.13	96.3 - 100.8	93.9 - 103.4	-0.16	0.13	3.14
	RL7			91.9	3.26	90.4 - 94.0	89.0 - 99.8	2.85	0.11	3.15
	RV7	5	2009	91.9	3.29	90.4 - 94.1	89.2 - 99.6	0.23	0.11	3.07
	RSA7			92.0	3.26	90.4 - 94.1	89.1 - 99.7	2.81	0.11	3.12
	RTIPS6	6	2009	97.4	3.32	94.5 - 101.4	86.9 - 106.9	-0.47	0.11	3.08
	RFW			31.4	3.55	27.2 - 34.7	16.6 - 38.5	4.71	0.12	2.96
	RL9		2010	73.1	3.26	69.3 - 77.3	60.5 - 82.2	-1.38	0.11	3.05
	RSA9			73.4	3.25	69.1 - 77.5	60.7 - 82.1	-1.59	0.11	2.94

Table B.1, continued.

	Trait[†]	LG[‡]	Env.	Peak[§]	LOD	LOD-1[¶]	LOD-2[¶]	a(H)[#]	$R^{2††}$	95% LOD
Root Size	RV9	6	2010	73.6	3.24	69.0 - 77.3	60.5 - 82.2	-0.15	0.11	2.96
	RL7			74.4	4.82	72.3 - 76.6	70.1 - 77.6	-2.97	0.17	3.01
	RSA7			74.4	4.82	72.2 - 76.9	70.2 - 77.7	-3.09	0.17	2.91
	RV7			74.4	4.76	72.2 - 77.0	70.2 - 77.5	-0.25	0.17	2.95
	RL3			95.4	5.12	93.4 - 95.4	89.6 - 97.7	13.37	0.17	3.03
	RSA3			95.4	5.47	93.3 - 95.5	89.5 - 97.6	3.09	0.18	3.17
	RV3			95.4	5.69	93.5 - 95.5	89.7 - 97.7	0.06	0.18	3.07
	RTIPS2	7	2009	7.1	4.72	5.9 - 8.2	1.6 - 15.2	7.84	0.17	3.04
	RTIPS4			17.7	3.46	13.4 - 19.3	9.4 - 22.3	0.78	0.12	3.10
	RL6	8	2010	25.2	3.32	16.5 - 32.1	12.7 - 37.8	-3.93	0.14	3.07
	RSA5			25.4	3.33	16.4 - 32.3	12.5 - 37.8	-2.75	0.14	3.16
	RV5			25.4	3.30	16.5 - 32.3	12.6 - 38.4	-0.15	0.14	3.19
	RL9			28.0	3.25	23.2 - 33.5	20.4 - 37.5	-1.04	0.11	2.99
	RSA6			28.1	3.78	24.4 - 36.1	21.8 - 39.8	-2.33	0.14	3.00
	RSA9			28.1	3.29	23.2 - 33.4	20.3 - 37.4	-1.39	0.11	2.93
	RV9			28.1	3.30	23.2 - 33.3	20.2 - 37.2	-0.15	0.11	3.07
	RL6			28.2	3.76	24.4 - 36.1	21.7 - 39.9	-2.66	0.14	3.03
	RV6			28.8	3.82	24.5 - 36.0	21.8 - 39.8	-0.16	0.14	2.99
	RSA5	10	2009	56.3	3.76	53.9 - 58.9	52.0 - 60.5	-3.60	0.13	3.18
	RL5			56.4	3.77	54.0 - 58.9	52.0 - 60.5	-5.09	0.13	3.10
	RV5			56.4	3.78	53.9 - 59.0	52.0 - 60.5	-0.20	0.13	3.32
	RDW		2010	61.5	3.84	59.5 - 63.1	58.0 - 63.8	-0.69	0.12	3.05
	RFW			63.1	4.69	61.7 - 63.6	59.5 - 67.1	-5.74	0.14	2.96
	RT7			63.2	4.82	62.6 - 63.9	56.8 - 64.0	-0.42	0.16	3.07

[†]Panicle fresh (PNFW) or dry weight (PNDW); shoot (S), stalk (STK), stem (STM), leaf (L), leaf sheath (LS), root (R), fresh (FW) or dry weight (DW); leaf length (L), width (W), or area (A); root length (L), surface area (SA), or volume (V); number of root tips (TIPS) or forks (FKS); total (T).

[‡]Linkage group.

[§]QTL peak in cM.

[¶]QTL width in cM at either 1 (LOD-1) or 2 (LOD-2) LOD units below the peak.

[#]Additive effect in units of the trait (flowering time (days), stalk length (cm), shoot biomass (g), panicle biomass (g), leaf length and width (cm), leaf area (cm^2), root length (cm), root surface area (cm^2), and root volume (cm^3).

[††]Proportion of total phenotypic variance explained by the QTL.

VITA

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