QTLS FOR ENERGY RELATED TRAITS IN A SWEET × GRAIN RIL SORGHUM [*SORGHUM BICOLOR* (L.) MOENCH] POPULATION

A Thesis

by

TERRY JOSEPH FELDERHOFF

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

MASTER OF SCIENCE

August 2011

Major Subject: Plant Breeding

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Approved by:

Chair of Committee, William Rooney
Committee Members, Seth Murray
Patricia Klein
Head of Department, David Baltensperger

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ABSTRACT

QTLs for Energy Related Traits in a Sweet × Grain RIL Sorghum [*Sorghum bicolor* (L.) Moench] Population. (August 2011)

Terry Joseph Felderhoff, B.S., Texas A&M University

Chair of Advisory Committee: Dr. William Rooney

Recent initiatives for biofuel production have increased research and development of sweet sorghum. Currently, the initial major limitation to integrating sweet sorghum into existing production systems is the lack of sweet sorghum hybrids adapted to industrial production systems. Hybrid development is now underway, and the application of genetic markers can be used to define the genetic basis of sugar yield and its components, as well as reduce the time required to deliver new sweet sorghum hybrids to market. The purpose of this research was to further characterize the genetic components that influence sweet sorghum productivity, agronomics, and composition. Specifically, a grain × sweet sorghum recombinant inbred line (RIL) population developed for quantitative trait locus (QTL) analysis related to sugar production was evaluated for 24 phenotypic traits including brix, percent moisture, and biomass yield across four environments. The 185 F_4 RILs were derived from the parents 'BTx3197' and 'Rio', which are pithy stalk grain and juicy stalk sweet sorghums respectively. Following screening, two genetic maps were constructed with 372 and 381 single nucleotide polymorphisms (SNPs) evaluated using an Illumina GoldenGate assay.

Analysis of the data in QTL Cartographer revealed a major and previously reported QTL for soluble solids on chromosome 3, but in contrast to previous studies, this QTL co-localized with other QTLs that have a negative influence on biomass and seed production. Therefore, selection for this QTL may not be advantageous. Because only a few QTLs for percent moisture were found, the results indicated that the pithy stalk phenotype does not have a major effect on percent moisture as measured in this study. Thus, breeding for high or low moisture content will be more challenging than previously expected. The absence of dominance effects indicated that brix must be high in both parents to produce high brix in the hybrid.

DEDICATION

I dedicate this thesis to my parents, who have supported me in more ways than one during my academic pursuits.

ACKNOWLEDGMENTS

I would like to thank my committee members for all their help, guidance, and rapid response to all my questions or revisions. Individually, I thank my committee chair, Dr. Rooney, for his part in not only this project, but also in shaping my path towards plant breeding, and my committee members; Dr. Murray, for his reminders that helped me get my project done on time, and Dr. Klein, for her input on the genetic portions of my project.

I would like to thank all the employees, graduate students, student workers, and visitors at Texas A&M Sorghum Research and Breeding for the wide range of help they gave on this project: Vickie Marriott, Karen Prihoda, Delroy Collins, Dustin Borden, Dr. Nilesh Dighe, Dr. Thomas Stefaniak, Dr. Dan Packer, Dr. Rebecca Corn, Miguel Gutierrez, Matt Bartek, Payne Burks, Leo Hoffmann Jr., Chad Hayes, Ostilio Portillo, Dr. Mohan Gowda, Michael Klepac Jr., Robin Jakubik, Jenna Jakubik, Josh Herrington, Catherine Lettunich, Brandon Reynolds, Pamela Benton, Alvaro França, Bradley Shelfer, Rafael Costa-Pavella, and Flavio Tardin.

I thank the Institute for Genomic Diversity at Cornell University and Dr. Martha Hamblin, Charlotte Acharya, Dr. Sharon Mitchell, and Dr. Stephen Kresovich. I thank the Institute for Plant Genomics & Biotechnology at Texas A&M University and Dr. Arun Sharma. I thank the Special Projects group at Texas AgriLife Research and Extension Center at Weslaco, TX. Financial support came from the United States Department of Energy through the DOE-USDA Feedstock Genomics Grant No. DE-FG02-07ER64458 (PI, Dr. Wilfred Vermerris, University of Florida).

NOMENCLATURE

ANOVA	Analysis of variance
BLUP	Best linear unbiased predictor
CIM	Composite interval mapping
CS	College Station, Texas
IM	Interval mapping
NIRS	Near-infrared spectroscopy
RCBD	Randomized complete block design
RIL	Recombinant inbred line
SM	Single marker
SNP	Single nucleotide polymorphism
QTL	Quantitative trait locus
WE	Weslaco, Texas

TABLE OF CONTENTS

Page

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

1. INTRODUCTION

Bioenergy has received considerable interest in the past decade as a new and renewable source of transportation fuel (Rooney et al., 2007). Bioenergy is produced from plant-derived biomass using conversion techniques such as thermochemical treatment and biochemical treatment (Antonopoulou et al., 2008). Thermochemical treatment involves direct combustion of biomass or the use of heat to transform biomass into intermediary gases or liquids that can be later converted to fuel (Wright and Brown, 2007). Biochemical treatment is a conversion technique that allows options for producing various types of fuel including ethanol, methanol, methane, and hydrogen (Demirbas, 2008). In the future, dedicated biomass and sugar crops are expected to account for a greater proportion of total bioenergy production, and this is currently most noticeable in ethanol production (Carpita and McCann, 2008).

Currently, ethanol is the most common bioenergy product and it is manufactured from many substrates and crops. Ethanol production in the U.S. is primarily derived from the starch in maize grain, while ethanol production in Brazil relies almost exclusively on sugar derived from sugarcane (Moschini et al., 2010). The widespread use of starch and sugar crops for ethanol production is due to the facts that these crops are high yielding, can grow in many countries, are relatively easy to convert to ethanol, and already have industrial infrastructure in place (Smith et al., 1987; Bothast and Schlicher, 2005).

Sweet sorghum is a potential bioenergy crop with similarity to sugarcane; it has

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This thesis follows the style of Crop Science.

high sugar concentrations in juice extracted from the stalk (Tarpley and Vietor, 2007). Sweet sorghum also has some agronomic advantages over sugarcane in that sorghum is seed-propagated instead of vegetatively-propagated, and it grows faster, reaching maturity in approximately four months compared to 10 to 12 months typical for sugarcane (Smith et al., 1987). Thus, sweet sorghum could be used as a complementary crop in existing sugarcane production (Burks et al., in prep). However, a major limitation to integrating sweet sorghum into existing production systems is the lack of sweet sorghum hybrids adapted to industrial production. Most traditional sweet sorghum varieties were developed primarily for syrup production for human consumption, and, for a variety of reasons, they are not optimized for industrial application. The design and development of sweet sorghum hybrids will make the crop much more viable for industrial applications.

The sorghum breeding industry is aware of these limitations and is actively working to mitigate them. Breeding sweet sorghum parental lines and hybrids require phenotyping that is new and unique to traditional sorghum programs. Phenotypic traits related to energy production are highly influenced by the environment, making accurate selections dependent on factors hard to control (Murray et al., 2008; Ritter et al., 2008; Shiringani et al., 2010). Phenotyping is slow and labor intensive, requiring hand harvesting and individual processing to access many of the desirable sweet traits. Many sweet sorghum genotypes have high lodging rates under conducive conditions, making phenotypic data harder to both collect, as well as accurately observe (Monk et al., 1984).

The application of molecular genetic techniques can help minimize these problems, effectively reducing the time required to deliver products to the commercial market, and providing a better understanding of the genetic basis of sugar yield and the components that influence this trait. One popular genetic technique is quantitative trait locus (QTL) mapping; once QTLs are identified, the flanking markers can subsequently be used in marker assisted selection of important traits (Fernando and Grossman, 1989).

QTL analysis of sweet sorghum has been previously reported using both linkage mapping (Natoli et al., 2002; Bian et al., 2006; Murray et al., 2008; Ritter et al., 2008; Shiringani et al., 2010) and linkage disequilibrium (association) mapping (Murray et al., 2009). These and other studies of sweet sorghum demonstrate nearly all phenotypic traits are highly affected by the environment, with juice and sugar yield being the most influenced (King et al., 1961; Ferraris, 1981).

Juiciness of the plant is determined by the percent moisture found in the plant. This trait is of importance because there are two major components when breeding for increased total sugar yield; juice yield and soluble solids (Corn, 2009). It has been reported that percent soluble solids has a physiological limit of ~25%, so breeding for increased juice yield is the most efficient method of increasing total sugar production (Mangelsdorf, 1958). Juice yield is mostly a quantitative trait, but one gene, *d*, that determines if a plant has pithy (dry) or juicy stems, has been reported (Hilson, 1916). Based on visual observations, it has been a standard assumption that pithy stems are drier (lower percent moisture) than those classified as juicy. Sweet varieties are almost

exclusively recessive for juicy stems; non-sweet sorghum for grain or forage vary for the trait with both juicy and pithy types being grown (Smith and Frederiksen, 2000).

Many juice and biomass traits advantageous for industrial use are positively correlated with plant height and maturity, as the plant has a greater chance to add biomass and store energy when it can grow taller and for a longer duration (Natoli et al., 2002; Murray et al., 2008; Ritter et al., 2008; Shiringani et al., 2010). Grain yield, generally low in sweet sorghum varieties, has not been found to be correlated with juice yield, but it has been negatively correlated with sugar concentrations (Murray et al., 2008; Ritter et al., 2008; Shiringani et al., 2010). Even though these correlations exist, very few grain yield and stem sugar QTLs have co-localized, and those that do have also co-localized with flowering time. Thus, they fail to explain the negative correlation between grain yield and sugar concentration.

The purpose of this research is to further characterize the genetic components that influence sweet sorghum productivity, specifically stem sugar and juice qualities, and confirm previously identified QTLs in independent genetic backgrounds and environments. Specifically, a pithy stalk grain × juicy stalk sweet sorghum recombinant inbred line (RIL) population was developed and investigated for traits related to sugar production. A high-density molecular map was developed to identify QTLs associated with energy production in sweet sorghum. The specific objectives of this study were to (1) identify QTLs for percent moisture as well as (2) QTLs for other traits directly related to sugar yield, such as juice yield, soluble solids, and composition of soluble

solids. In addition, (3) QTLs for other agronomic traits were identified and (4) dominance effects for all traits were examined.

2. MATERIALS AND METHODS

2.1 Population Development and Family Structure

An RIL population of 185 F_4 lines was derived from the parents 'BTx3197' and 'Rio' for this study. Rio, the male parent line, is a juicy stalk sweet sorghum variety (Broadhead, 1972). It has been used as a parent line in many high yielding sweet sorghum varieties to date (Murray et al., 2009). Rio has a moderate level of photoperiod sensitivity which results in a moderately delayed flowering time during long days (~100 days after planting), substantial height (~260 cm), and has the ability to produce a ratoon crop in environments with long enough growing periods (Broadhead, 1972). The sugar and biomass yields of Rio are comparable to standard sweet varieties, yielding an average sugar yield of 3.6 t ha^{-1} and producing 17.5 Mg ha^{-1} of total dry biomass (Ferraris, 1981). BTx3197 is a derivative of combine kafir SA 5765-10-2, a pithy stalk grain sorghum, and was released by the Texas Agricultural Experiment Station in 1950 (King et al., 1961; Smith and Frederiksen, 2000). BTx3197 has good general combining ability, a mid to early flowering time (~70 days after planting), and it was one of the early seed parents for hybrid grain sorghum (King et al., 1961).

2.2 Experiment and Field Layout

The RILs and the parents were planted in multiple environments in a randomized complete block design (RCBD) with two replications per environment. In 2009, the trial was grown in two environments in College Station, TX; one planted on April 1st which is the standard planting date for this location. The second trial in 2009 was planted on June 16th to represent a late season harvest, which is important for continual harvest in a

production system. In 2010, the trials were planted in Weslaco, TX on February 17th, and in College Station on April 6th; both of these dates are common times for sorghum planting in their respective locations.

Standard sorghum agronomic practices were used at all locations, with supplemental irrigation used to ensure consistent and high yields. In College Station the plots were 5.5 meters long spaced apart on 0.76 meter intervals; in Weslaco, each plot was 5.18 meters long spaced 1.02 meters apart. Rolling cultivation was performed on the College Station fields in the early stages of growth, followed by sweep cultivation immediately after side-dress fertilization at the five leaf stage. The College Station fields were fertilized with 330 kg ha^{-1} of 10-34-0 and 22 kg ha^{-1} of zinc prior to planting, and side dressed with 175 kg ha^{-1} of 32-0-0 after the first cultivation and before sweep cultivation. For Weslaco, the fields were fertilized with 470 kg ha^{-1} 4-10-10 plus 4.68 l ha^{-1} Quick Boost with Awaken. Herbicides applied were 4.68 l ha^{-1} of Atrazine 4L and 1.52 l ha^{-1} of Acetamide to College Station, and 2.43 l ha^{-1} Atrazine 4E applied at Weslaco. To control insect pests, the late planting in College Station 2009 also received insecticide (Karate) at the rate of 0.73l ha^{-1}. The soil type for College Station is Raymondville Clay Loam, and for Weslaco is Ships Clay Loam (Corn, 2009).

2.3 Phenotyping and Harvesting

Field notes were taken on the following agronomic traits prior to harvest: height, exertion, lodging, and flowering time. Plant height was measured prior to harvest as an average for the whole plot in centimeters (cm) from the ground to the top of the panicle. Exertion was measured at the same time as height and is defined as the length of the

peduncle from the flag leaf to the base of the panicle. Severity of stalk lodging was estimated on a scale of 1-9; 1 representing no lodging to 10% lodging, 9 representing 90% lodging to complete lodging, and each interval in between representing a 10% increase. Flowering time was estimated by recording the number of days from planting to when 50% of the plot reached mid-anthesis; it was assumed that grain fill duration was consistent among the genotypes and predicts physiological maturity.

Plots, with the exception of the most extreme photoperiod sensitive lines, were harvested within two days of physiological maturity, which was determined by visual observation combined with flowering time described previously. Physiological maturity is the commonly reported time for maximum sugar yields in sweet sorghum (Lingle, 1987; Almodares et al., 2007). At harvest, a one meter section of the plot was hand harvested, and post-harvest data was collected from this sample. The harvested subplots were phenotyped immediately following harvest for the following traits: harvest yield, vegetative yield, panicle yield, juice yield, and brix. Harvest yield was measured in kilograms on an Ohaus Defender 5000 series digital scale (Ohaus Corp.; Pine Brook, NJ USA), then the panicles were cut from the stalks, and the stalks were weighed to measure vegetative yield. The difference between harvest yield and vegetative yield was recorded as panicle yield. The extraction of juice was performed with a portable three-roller Ampro Sugarcane Crusher diamond model (Ampro Exports; New Delhi, India), and a sub-sample of both the juice and the bagasse was collected. Juice yield was measured in grams on an Ohaus Adventurer digital scale. Brix, a measure of the percent soluble solids in a liquid, was measured for the juice using an Atago PAL-1 digital

pocket refractometer (ATAGO Co., LTD; Itabashi-ku Japan) with a range of 0-53%. A 15 ml juice sample was collected and treated with Bussan 881 biocide, to prevent degradation of composition, and subsequently frozen for storage. A bagasse sample was pulled and weighed on an Ampro Adventurer digital scale, then dried in a Despatch forced convection drier (Despatch Industries; Minneapolis, MN USA) for approximately four days at 51-57°C, and re-weighed to extrapolate residual sample moisture. The dried bagasse was then ground using a Wiley standard model 3 knife mill with a 2 mm sieve (Arthur H. Thomas Co.; Philadelphia, PA USA), and stored for later analysis.

2.4 Compositional Analysis

Compositional analysis was performed on both the ground bagasse and the juice samples by near-infrared spectroscopy (NIRS) using a FOSS XDS MasterLab, with the XDS Rapid Content and XDS Rapid Liquid modules for the bagasse and juice samples, respectively (FOSS NIR Systems Inc.; Laurel, MD USA). The samples were scanned at 2 nm wavelength intervals, ranging from 400 to 2500 nm, using FOSS ISI-scan software. Each sample was scanned at least twice to assure an accurate reading, with a third scan taken on a random 10% of the samples for confirmation of accuracy.

Cellulose, lignin, protein, sucrose, glucan, xylan, arabinan, galactan, and ash concentrations were predicted for the bagasse samples using FOSS Win-ISI software and the predictive curve developed by the Texas A&M Sorghum Lab and NREL (Wolfrum et al., personal contact, in prep). All the traits are predicted as a sum of a whole, so individual traits are recorded in percent and all composition traits combined add up to approximately 100%. The regression curve for liquid samples was developed using juice

samples from this, and other, experiments through a collaboration between the Texas A&M Sorghum Lab and the Chambliss Research Group in the Baylor University Department of Chemistry.

2.5 Experimental and Statistical Analysis

All collected data were used for QTL analysis by determining least squares for each location across replications, as well as best linear unbiased predictors (BLUPs) calculated across all locations. BLUPs were used when combining data from all locations since this type of statistical analysis is more comparative than least square means, as it takes into consideration different ranges for random model effects. Therefore, the phenotypic value of a trait that is high in a favorable environment will not have an overwhelming effect when combined with data from a more stressful environment. Data was analyzed with all random effects in an analysis of variance (ANOVA) test using fit model in JMP 8.0 (SAS Institute Inc.; Cary, NC USA).

2.6 Genotyping, Map Construction, and QTL Analysis

Leaf punches were collected from four random seedlings per line and bulked. The punches were freeze dried for DNA extraction and subsequent marker analysis. The tissue was sent to Cornell University, where DNA was extracted and genetic markers were generated using a 1536 single nucleotide polymorphism (SNP) array with the Illumina GoldenGate (Illumina Inc.; San Diego, CA USA) sorghum assay developed at Cornell University. Tissue from each line is in the F_4 generation, but because the tissue was bulked from multiple plants the genotypes, like the phenotypes observed, represent the F_3 parent. This was expected to make the QTL analysis less accurate when analyzed

as an RIL population, but gave the opportunity to assess dominance effects when analyzed as an F_3 population.

A total of 488 markers and 185 RILs were available for the creation of the two genetic maps. The genetic maps were constructed using MAPMAKER/EXP in conjunction with JoinMap, first as an RIL population by treating heterozygous markers as missing data, then as an F_3 population in order to predict dominance effects, taking into account both the homozygous and heterozygous loci (Lander et al., 1992). The markers were placed in order and assigned physical distances based on the BTx623 sorghum genome sequence assembly (Paterson et al., 2009). The marker data was analyzed in MAPMAKER/EXE to both verify marker order, as BTx623 is not a parent used in this population, and to obtain centimorgan (cM) distances between markers for later QTL analysis. When the cM distance between adjacent markers was 0.3 or less, the physical position of the markers was used to determine marker order.

The genetic linkage maps and phenotypic data were imported into QTL Cartographer to identify significant QTL for the traits analyzed (Wang et al., 2007). Each trait was run independently for each environment and all environments were combined using BLUPs and analyzed. The genetic association of the phenotypic data was analyzed using three different methods: single marker (SM) analysis, interval mapping (IM) analysis, and composite interval mapping (CIM) analysis. Combined with the RIL population and the F_3 population maps, this gave a total of six different QTL tests. Simulations have shown that comparing different analyses can be useful for determining robustness of the data in decision making (S.C. Murray, personal

observation). To establish a significance threshold for each QTL, the data was permuted 1000 times at a confidence level of alpha 0.05, and a walk speed of 1 cM. Only QTLs at or above the LOD threshold for a given trait are reported. The size of a given QTL was determined as the width at two LOD units below the QTL peak and neighboring QTL were required to be a minimum of 5 cM apart at their peaks.

3. RESULTS AND DISCUSSION

3.1 Phenotypic Performance and Analysis

Significant differences in phenotypic performance between the two parents were observed for most measured traits (Table 1). Among the RILs, transgressive segregation was seen for most traits, though for a few traits the value observed for BTx3197 was lower than any progeny (Table 1). This was most evident for plant height because BTx3197 is homozygous for height genes dw_1, Dw_2, dw_3, dw_4, and Rio is believed to be homozygous for dw_1, Dw_2, Dw_3, dw_4 (Klein et al., 2008; W.L. Rooney, personal observation). Thus the progeny will always have a dominant Dw_2 height gene, and will never be shorter than the BTx3197 parent due to alleles at the *dw* loci.

The flowering times in Weslaco were later, and had a much narrower range, than flowering times observed in other environments (Table 1). This is not unexpected since the planting took place when day lengths were less than 12 hours and any photoperiod-sensitive response in the RILs is eliminated in this environment. In addition, all genotypes' growth was slower due to less total heat and light in the late winter.

The College Station planting in June 2009 had lower yields for almost all data points collected (Table 1), primarily because temperatures were cooler and wetter throughout the fall, which resulted in significant lodging and lower growth rates (data not shown). These effects jointly explain why this environment was not ideal for sorghum growth, and provides a stressful environment for comparison purposes. Data from this environment was still proven to be predictable and effective for analysis of traits, but the information gleaned is still approached with caution.

3.2 Genetic Map

A total of 372 polymorphic markers were used to create a genetic map using an RIL data type in MAPMAKER/EXP, consisting of 11 linkage groups that spanned 1246 cM (Figure 1). The marker orders along the genetic map align almost perfectly with the marker orders projected using the sorghum physical map (Figure 1). The distribution of markers is comparable with other QTL studies, having areas of clustered markers and gaps of no markers (Mace and Jordan, 2010; Mace and Jordan, 2011). Only chromosome 6 was separated into multiple linkage groups (Figure 1). This grouping is supported by the physical map, with the last marker on group 6A being 20 million base pairs away from the first marker on group 6B.

A second genetic map using data type F_3 in MAPMAKER/EXE was created using 381 markers. The map spans 3359 cM and includes ten linkage groups (Figure 2). The distribution of markers along the map is relatively consistent with previous mapping studies, and has no extremely tight clustering of markers (Mace and Jordan, 2010; Mace and Jordan, 2011). The marker orders are closely aligned with the expected marker orders based on the sorghum physical map, and because there were no extremely clustered markers the physical order of markers was seldom used to alter the marker order on the map (Figure 2).

When comparing the two maps, the F_3 map is more evenly spaced than the RIL, yet still retains the same overall groupings and gaps (Figure 1; Figure 2). Comparing the marker orders to the physical map, the markers on the RIL map were strongly linked to the physical marker order, while the F_3 map had several markers out of order. Although

all 488 available markers were used when generating both maps, markers were removed during the creation process for multiple reasons; failure of markers to link with groups, markers with too many missing data points, and markers that, when mapped, where deemed too inconsistent with the physical map order. Many of the same markers were removed from both tests during analysis, with slightly more removed in the RIL than in the F_3.

The proportion of heterozygous markers was lower than the predicted 25%, averaging 20.1% with a range of 7% to 33.3%. The proportion of BTx3197 to Rio alleles were similar (BTx3197 mean = 45.2%; Rio mean = 49.9%), with segregation distortion seen in two regions. The first involved 13 markers on chromosome 1 with an average of 29.4% of the lines with a BTx3197 allele; the second was a block of six markers on chromosome 7 with an average of 27.1% RILs having the BTx3197 allele. The distortion on chromosome 7 aligns with the major height gene, *Dw*$_3$; a distortion that has been seen previously (Menz et al., 2002; Murray et al., 2008; Mace and Jordan, 2010). The distortion on chromosome 1 encompasses a major effect photoperiod gene, and this distortion may derive from the selfing methodology used to develop the RILs (Mace and Jordan, 2010).

As expected, the RIL and F_3 maps are similar to each other and to other genetic maps previously reported (Mace et al., 2009). The slight differences in the marker distribution of the two maps can be explained by the increase in data points from heterozygous markers included in the F_3 map. In the RIL map, chromosome 6 is divided into two linkage groups due to a lack of SNPs in a ~20 million base pair region, and it is

likely a region of agronomic traits that have been selected for identically between the parents. This region encompasses the *Dw*$_2$ height gene for which both parents are homozygous dominant (Mace and Jordan, 2010; W.L. Rooney, personal observation).

3.3 QTL Analysis

Of the six methods used for QTL detection, the greatest number of significant QTLs was seen when the population was treated as an F_3 and the data analyzed by SM analysis; this is primarily due to multiple linked markers being identified as significant whereas in IM or CIM mapping, these regions are typically classified as a single QTL in a genomic region (Table 2). The F_3 population on a whole has a higher LOD rating for QTLs than the RIL population which implies greater significance of QTL analysis (Table 2). This might be due to the fact that analyzing the population as an F_3 would be more representative of the actual population genotyped and phenotyped, including dominance effects, less missing data in the model, and predicted recombination events. The R^2, the amount of explained variation, is highest in the F_3 population with the F_3 IM having the highest average R^2 (Table 2).

A reality of QTL studies is the possibility of identifying false QTLs (Type I error) and failing to identify true QTLs (Type II error) (Bernardo, 2004). Analyzing the data using six different methods can help to reveal which QTLs are robust to the error of a single model. The number of QTLs shared between the methods is used to find which method are the most informative and which QTLs are deemed false (Table 2). The F_3 IM is the poorest model at explaining the QTLs seen in other models, mainly due to the small number of QTLs identified, while F_3 CIM analysis results in the most unique

QTLs, while still sharing many QTLs in common with the other methods (Table 2). The CIM for the RIL population is the best visual representative method, as it accounts for the greatest percentages of QTLs seen in all other analyses. For this reason, the results from analyzing the population as an RIL with CIM are displayed in Figure 3 (Table 2).

3.4 Agronomic QTLs and Brix QTLs

As expected, QTLs were detected for all of the agronomic traits measured in this study (Table 2; Figure 3). Most of the QTLs detected herein have been reported in previous studies (Mace and Jordan, 2011). These basic agronomic QTLs often have large effects, and mask smaller QTLs for numerous other traits. The height and flowering time QTLs are the best example of this with height QTLs always co-localizing with at least 10 other traits (Figure 3). The two main height QTLs co-localize with major height genes Dw_3 (chromosome 7) and *Sb.Ht9.1* (chromosome 9) (Mace and Jordan, 2010). The QTL for height on chromosome 4 is not associated with any known location of major height genes, and could be a product of the flowering time QTL that shares the same location. Not only does this flowering time QTL co-localize with height, but also the flowering time QTL on chromosome 6 co-localizes with biomass and juice yield traits and the major maturity gene, Ma_1 (Mace and Jordan, 2010).

Among the QTLs found in this study, the the brix QTL on chromosome 3 was consistently the most significant with regards to the highest R^2 and LOD score. This QTL was previously identified for brix and sucrose by Natoli et al. and Murray et al. (2002; 2008; Figure 3). This QTL was expected for a number of reasons: the parents were on opposite extremes for brix, were either the same (Rio) or related (BTx3197) to

the parents used by Murray et al. (2008), and the harvest times were implemented to capture optimum stem sugar concentrations. This QTL is seen in at least three environments in five of the different analysis methods, with the only exception being the F_3 SM test. Two other QTLs were predicted for brix; one on chromosome 1 and the other on chromosome 2. Both were seen in more than one environment, and were seen consistently across the different analysis methods.

The chromosome 3 brix QTL detected in this study confirms previous reports by Murray et al. (2008), but it is also contradictory to their conclusion that breeding for this QTL lead to increased sugar production without compromising other desired traits (Murray et al., 2008). In the original report, no other trait co-localized with this QTL, but in the present study, there are many other QTLs co-localizing with this QTL, including vegetative weight, total biomass, and even percent moisture (Figure 3). Thus, breeding for this QTL will increase brix, but that increase may come at some reduction in other traits. Identifying the genetic basis underlying this QTL would be valuable for understanding the mechanics of this trait, as well as to delineate between pleiotropy and linkage.

3.5 Juice QTLs and Pithy Stalks

Percent moisture showed transgressive segregation among the progeny, and was moderately heritable (Table 1). The largest QTL for percent moisture was located on the end of chromosome 1; it was detected in most of the analyses (Figure 3). Interestingly, this QTL did not co-localize with any other traits, was increased by the Rio allele, and was observed only in the June planting of 2009 College Station. Given the difference of

this environment from the other three, it is logical to question whether or not this QTL is a product of a specific environment, or if nuances of this environment had better resolution to map this QTL. Another QTL is seen on chromosome 2, this one, interestingly, is increased by the BTx3197 allele. The last QTL seen for percent moisture is on chromosome 3, co-localizing with a QTL seen for brix.

For juice yield itself, a QTL is on chromosome 1, which is detected in multiple locations and in almost every analysis method, and does not co-localize with agronomic traits. There is a QTL on chromosome 3 which co-localized with the major brix QTL. However, this QTL may not be a product of increased solute production, but instead caused by decreased juice yield, which has been shown to increase the brix value (Corn, 2009).

Overall, none of the QTLs for percent moisture were detectable across all environments. This was somewhat unexpected because BTx3197 has a pithy stalk while Rio has a juicy stalk and this trait has historically been considered a simply inherited trait (Hilson, 1916). Pithy and juicy stalks are easily differentiated visually, and it has been assumed that there is a difference in moisture and juice extraction rate between the two, but that has never been quantified. Given these assumptions, high definition QTLs were expected because of the parents' stalk phenotypes and the range in percent moisture in the RILs (Table 1). However, the difference in percent moisture between BTx3197 and Rio is narrow and no statistical difference existed between them. In fact, in several environments the percent moisture of BTx3197 was actually higher than Rio (Table 1). Thus, with little variation between the parents, few QTLs were detected across

the methods used to evaluate moisture. Based on this information, we conclude that pithy stalk does not necessarily correlate to a lower moisture content, and breeding for an increase in percent moisture will require using quantitative small effect genes for a long period of time.

3.6 NIRS Results and Composition QTLs

QTLs were detected for numerous compositional traits in this study (Table 2; Figure 3). Several of the QTL composition traits co-localized with other traits and almost all were increased as measured by BTx3197 alleles (Figure 3). In the bagasse samples, a total of 56 QTLs were identified for cellulose, lignin, hemicellulose, protein, and residual sugar. Of these QTLs, only one on chromosome 4 for galactan, a component of hemicellulose, appeared in all environments.

Major QTLs for both cellulose and lignin were detected on chromosome 3; the cellulose QTL was identified in three environments whereas the lignin QTL was only identified in two environments (Figure 3). Both of these QTLs co-locate with several other QTLs detected on chromosome 3 including the brix QTL (Figure 3). A lignin QTL on chromosome 7 co-localizes with an allele for decreased height (Figure 3). Thus, breeding for increased height and brix could actually lower lignin concentration as well as other structural components, given the highly significant ($p < 0.001$) negative correlation between these traits (Table 4). Lower concentrations of structural components combined with tall plants typically results in significant lodging if these relationships are not carefully managed during the selection process (Pedersen et al., 2005).

Sucrose is the only component for which increases in concentration were the result of alleles derived entirely from Rio (Figure 3). Given that Rio is a sweet sorghum, and that sucrose in the stem correlates to brix, these results are not unexpected (Natoli et al., 2002; Murray et al., 2008; Ritter et al., 2008; Corn, 2009; Shiringani et al., 2010). Therefore, co-localization of sucrose and brix QTLs on chromosome 3 was not surprising (Figure 3). It is interesting to note that the QTL for sucrose on chromosome 7, which was seen in multiple environments and consistently in multiple analysis methods, did not co-localize with a brix QTL in this study (Figure 3). However, in the QTL analysis performed by Murray et al. (2008), a QTL for both brix and sucrose was found on chromosome 7, however they concluded this to be a pleiotropic interaction from a height QTL. Sucrose also has a significant negative correlation with all other composition traits, though it is possible that because the composition traits are reported as percentage of the dry sample, an increase in a sample's residual stem sugar would appear as a decrease in other components, but this study was not designed to separate these composition traits.

3.7 Dominance Effects

Examination of the dominance effects of the traits measured revealed that all plant height QTLs were partially dominant, while all exertion and flowering time QTLs were completely dominant (Table 3). Harvest and vegetative yield QTLs were completely dominant, while panicle yield was primarily an additive trait. For brix, the QTL on chromosome 1 had strong partial dominance, and the QTL on chromosome 3 was additive. All other small QTLs for brix were additive as well. The QTL for percent

moisture on chromosome 1 was observed only in the SM analysis, and dominance effects cannot be measured in SM analysis (Liu, 1998). The QTLs that were found for percent moisture with F_3 IM and CIM were too inconsistent to predict any effects. Juice yield, although lacking any strong QTLs, was over-dominant or partially dominant for the QTLs predicted. The results imply that high juice yield in one parent will produce hybrids with high juice yield, but both parents should be high in brix if high brix concentrations are desired in the hybrid. Though effects are overestimated in QTLs, the dominance effects are proportional to the additive effects, and predictive of the true type of dominance (Xu, 2003).

4. CONCLUSION

A major effect QTL for brix was detected on chromosome 3 as has been reported previously, but it was also undesirably co-localized with other traits for bioenergy production. Consequently, selection for brix at this locus may result in an associated reduction in biomass yield as well. The moisture content of the plant is not simply inherited and the presence of pithy stalk is not a good indicator of percent moisture or juice yield as was previously believed. Percent moisture is heritable, but it is a quantitative trait that requires additional considerations and evaluation. Compositional analysis has shown that many composition traits are linked with other agronomic traits, and that selection of one these traits can inadvertently affect others if they are not consistently observed during the selection process.

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APPENDIX

Table 1. Phenotypic trait data for BTx3197, Rio, and 185 derived RILs evaluated in four Texas locations.

Trait	College Station April 2009				College Station June 2009			
	BTx3197	Rio	RIL Mean[†]	RIL Range	BTx3197	Rio	RIL Mean[†]	RIL Range
Height cm	104.9	238.3	197.9 (20.3)	143.5-256.5	na[‡]	na[‡]	na[‡]	na[‡]
Exertion cm	8.4	7.1	11.2 (4.1)	2.5-31.8	na[‡]	na[‡]	na[‡]	na[‡]
Flowering time d	69	91	75 (2)	65-87	67	82	70 (3)	61-83
Lodging	1	2	3 (2)	1-7	na[‡]	na[‡]	na[‡]	na[‡]
Harvest yield Mg ha^{-1}	26.8	74.5	57.2 (12.5)	28.6-95.2	20.1	45.5	40.6 (13.6)	15.2-83.1
Vegetative yield Mg ha^{-1}	22.6	70.2	51.6 (11.4)	25.1-87.8	18.3	43.1	37.6 (12.6)	13.7-79.1
Panicle yield Mg ha^{-1}	4.3	4.3	5.5 (1.7)	1.8-11.6	1.7	2.4	3 (1.4)	0.9-7
Percent moisture A %[§]	21.4	24.4	24.7 (5.1)	11.1-34.5	20.8	21.9	26.5 (5.2)	12.2-37.8
Percent moisture B %[¶]	74.4	69.1	71.7 (3)	63.6-78.5	75.9	74.0	75.1 (2.2)	69-81.8
Dry biomass A Mg ha^{-1}[#]	17.9	52.7	38.7 (8.3)	20.8-64	12.6	33.1	27.4 (8.9)	8.9-49.9
Dry biomass B Mg ha^{-1}[††]	5.8	21.5	14.7 (3.5)	7.1-26.6	3.9	11.0	9.4 (3.2)	3.3-21.6
Juice yield Mg ha^{-1}	4.7	17.5	12.9 (4.2)	4.3-26.3	3.5	10.2	10.2 (4.4	2.2-29.1
Juice by height	8.7	14.2	12.5 (3.9)	4.3-21.4	na[‡]	na[‡]	na[‡]	na[‡]
Brix %	10.8	18.1	16.5 (1.3)	12.3-19.7	8.7	14.4	11.9 (1.1)	8.7-15.3
Sugar yield Mg ha^{-1}[‡‡]	0.5	3.2	2.1 (0.7)	0.6-4.6	0.3	1.5	1.2 (0.6)	0.2-4.4
Extraction efficiency %[§§]	28.8	35.1	34.4 (6.7)	16.3-48.3	27.4	29.5	35.2 (6.9)	17-51.8

Trait	College Station 2010				Weslaco 2010			
	BTx3197	Rio	RIL Mean[†]	RIL Range	BTx3197	Rio	RIL Mean[†]	RIL Range
Height cm	103.6	337.8	252 (26.7)	149.9-355.6	133.4	225.6	211.1 (15)	155.7-260.4
Exertion cm	8.4	11.4	11.4 (3.8)	2.5-29.2	14.2	17.0	19.1 (3.8)	10.2-32
Flowering time d	na[‡]	na[‡]	na[‡]	na[‡]	85	89	87 (2)	82-92
Lodging	1	2	2 (2)	1-8	1	1	1 (1)	1-5
Harvest yield Mg ha^{-1}	26.4	81.6	57.5 (17.4)	19.4-106.3	25.4	48.6	40.6 (8.3)	15.5-75.6
Vegetative yield Mg ha^{-1}	23.4	76.6	54.3 (16.6)	17.8-103.1	19.3	44.4	35.4 (7.1)	13.7-69.4
Panicle yield Mg ha^{-1}	2.8	2.0	3.3 (1.6	0.4-7.4	6.2	4.2	5.3 (1.8)	1.8-10.5
Percent moisture A %[§]	15.1	22.5	22.4 (3.7)	12.3-34.6	28.6	31.7	32.5 (6.2)	18.7-42.1
Percent moisture B %[¶]	78.6	69.9	76.3 (2.8)	67.7-86.5	76.7	75.5	77.4 (3.3)	67.3-82.5
Dry biomass A Mg ha^{-1}[#]	19.7	59.4	42 (12.3	20.9-77.2	13.8	30.3	23.9 (5.4)	9.9-46.1
Dry biomass B Mg ha^{-1}[††]	5.0	23.1	12.5 (3.7	6.1-25.7	3.9	10.9	8 (2)	3-15.8
Juice yield Mg ha^{-1}	3.6	18.0	12.5 (4.9)	3.1-28.5	5.4	14.1	11.5 (3.3)	3.7-23.3
Juice by height	6.7	10.3	9.4 (3.6)	2.8-18.9	10.5	16.2	14 (3.9)	6-26.5
Brix %	9.6	16.9	14.3 (1.6)	6.8-17.9	9.0	16.6	13.8 (1.4)	9.9-17.9
Sugar yield Mg ha^{-1}[‡‡]	0.3	3.1	1.8 (0.8)	0.5-4.5	0.5	2.3	1.6 (0.5)	0.5-3.1
Extraction efficiency %[§§]	19.1	32.2	28.5 (4.8)	16.4-44.2	36.0	42.1	41.8 (7.8)	24.6-54.3

[†]Standard error reported in parenthesis.
[‡]Data not available.
[§]Percent moisture A = (vegetative yield - dry biomass A) /vegetative yield
[¶]Percent moisture B = (vegetative yield - dry biomass B) / vegetative yield
[#]Dry biomass A = vegetative yield - juice yield
[††]Dry biomass B = (vegetative yield - juice yield) × (pressed stem weight dry / pressed stem weight wet)
[‡‡]Sugar yield = juice yield × brix
[§§]Extraction efficiency = juice yield / (vegetative yield - dry biomass B)

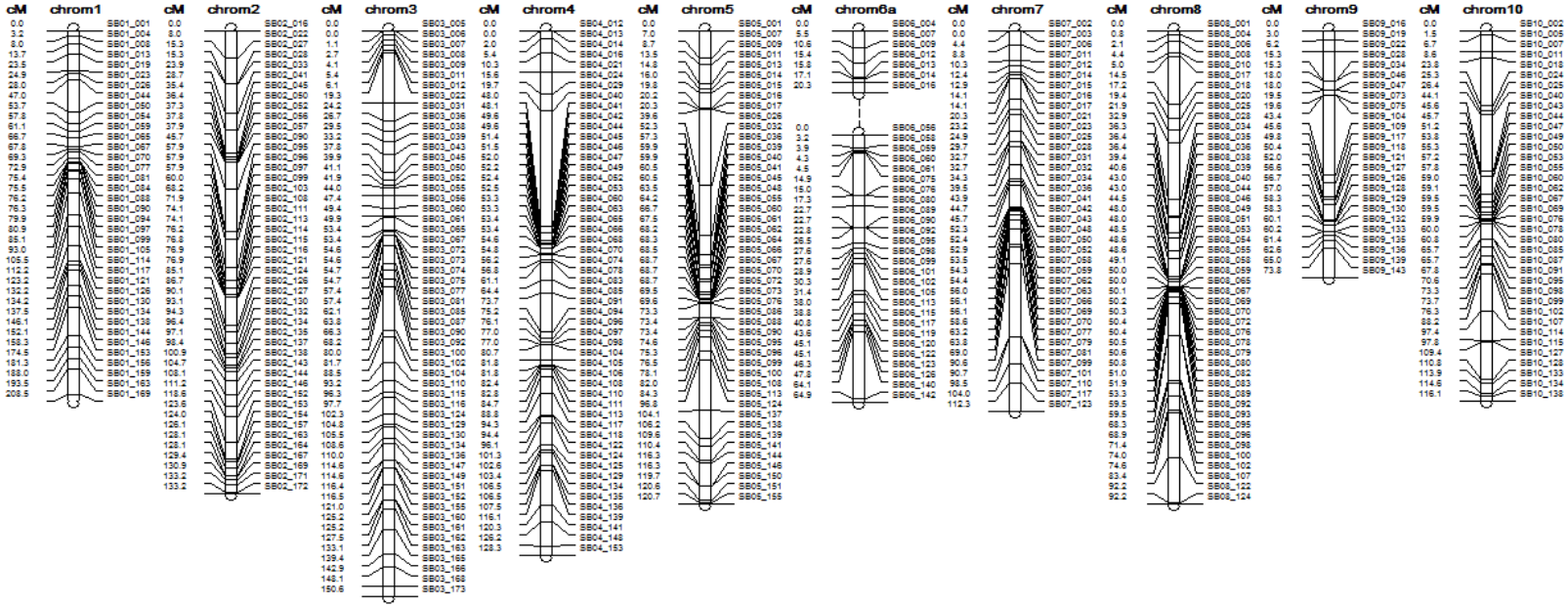

Figure 1. Genetic map derived from the BTx3197 × Rio population assuming entries are RILs. Markers are labled by their respective chromosomes and order on said chromosome as determined by the BTx623 sorghum genome sequence assembly (Paterson et al., 2009).

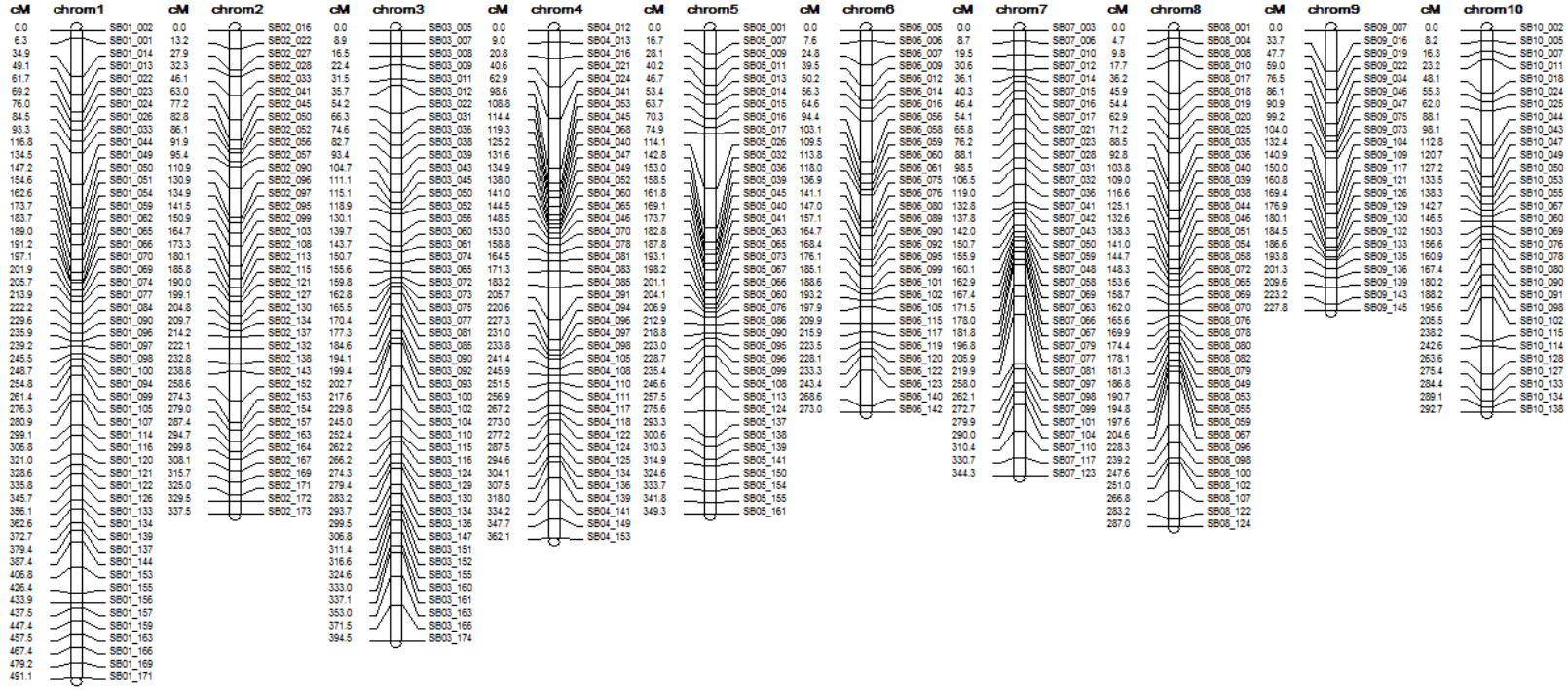

Figure 2. Genetic map created from the BTx3197 × Rio population analyzed as an F_3 population. Markers are labled by their respective chromosomes and order on said chromosome as determined by the BTx623 sorghum genome sequence assembly (Paterson et al., 2009).

Table 2. Comparison of data collected between single marker analysis, interval mapping analysis, and composite interval mapping analysis for both RIL and F_3 testing methods for 17 phenotypic traits.

Statistic	RIL			F_3		
	SM	IM	CIM	SM	IM	CIM
Number of QTL	95	73	109	125	53	113
Mean of LOD (SD)[†]	3.28 (0.9)	3.55 (1.1)	4.22 (1.3)	3.48 (1)	4.81 (1.5)	4.37 (2.5)
Mean of R^2 (SD)[†]	na[§]	0.101 (0.04)	0.095 (0.03)	na[§]	0.153 (0.05)	0.123 (0.06)
Correlation						
Seen in RIL SM (%)[‡]	--	54 (74)	52 (47.7)	58 (46.4)	28 (52.8)	38 (33.6)
Seen in RIL IM (%)[‡]	52 (54.7)	--	43 (39.4)	61 (48.8)	30 (56.6)	29 (25.7)
Seen in RIL CIM (%)[‡]	60 (63.2)	47 (64.4)	--	61 (48.8)	30 (56.6)	58 (51.3)
Seen in F_3 SM (%)[‡]	58 (61.1)	49 (67.1)	50 (45.9)	--	32 (60.4)	54 (47.8)
Seen in F_3 IM (%)[‡]	30 (31.6)	27 (37)	28 (25.7)	40 (32)	--	38 (33.6)
Seen in F_3 CIM (%)[‡]	51 (53.7)	35 (47.9)	52 (47.7)	60 (48)	40 (75.5)	--

[†]Standard deviation reported in parenthesis.
[‡]Percent the shared QTLs consist of the total QTLs is reported in the parenthesis.
[§]Data not available because single marker analysis does not report R^2.

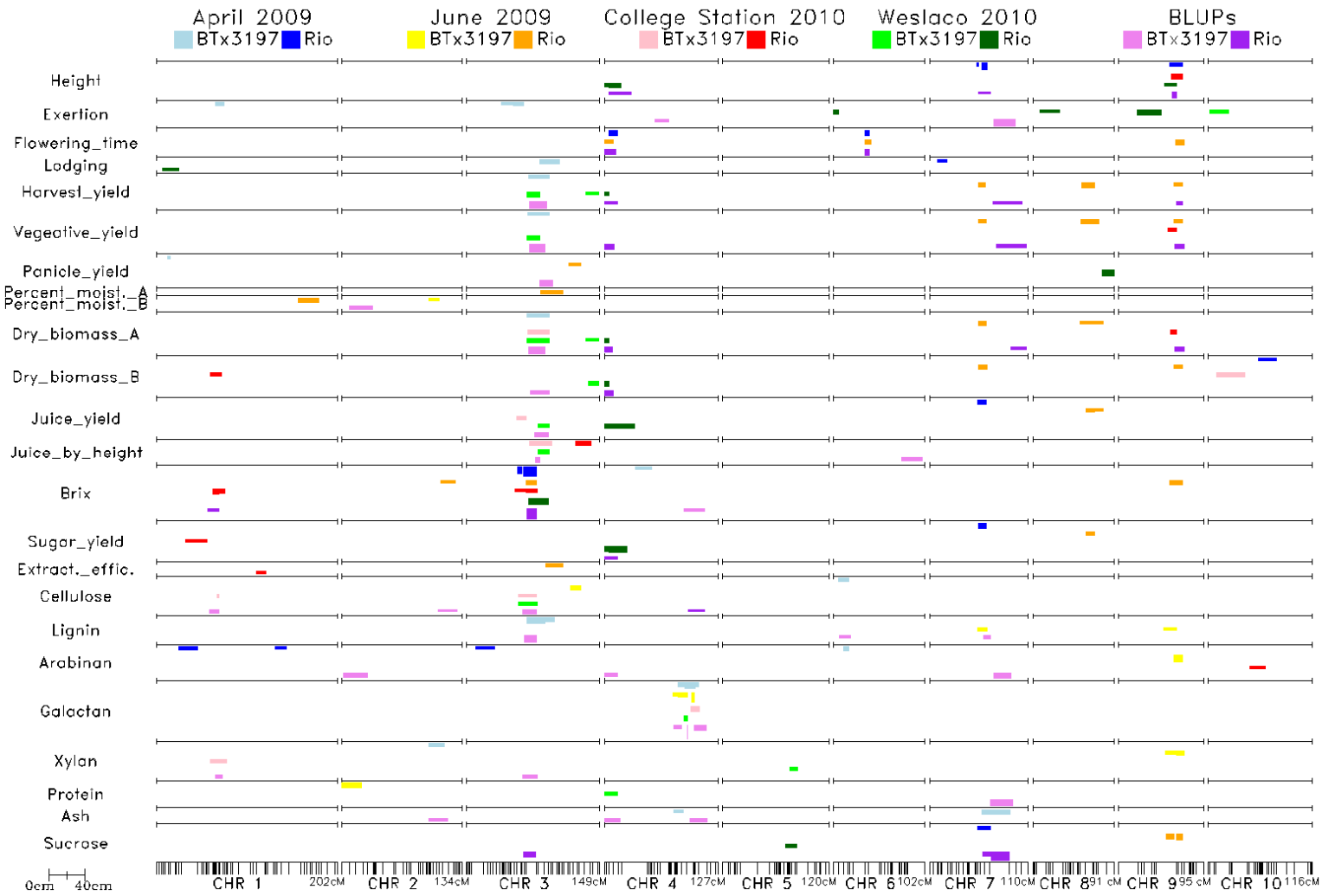

Figure 3. Estimated locations of QTL for traits measured during 2009 and 2010, and BLUPs across locations estimated from the BTx3197 × Rio RIL population using an RIL CIM. The color of the bar denotes the environment of the QTL, and which parental allele increased the trait. The width of the bar estimates the region the QTL covers at its 2-LOD interval, while the height of the bar is relative to the $R^2$of the QTL; a taller bar means a higher R^2.

Table 3. Additive (add) and dominant (dom) effects for phenotypic traits from F_3 CIM QTL analysis. The signs of the additive effects delineate which parent provides the increasing allele; a positive sign means the BTx3197allele increased the phenotypic value of the trait, and a negative sign means the BTx3197 allele decreases the phenotypic value of the trait, and thus the Rio allele will increase the phenotypic value.

Trait	Chrom	Location	Add	Dom
Height	4	WE 10	-4.	2.5
Height	4	BLUP	-2.3	1.2
Height	6	CS Apr 09	-1.1	-6.2
Height	6	CS 10	-6.2	1.8
Height	7	CS Apr 09	-4.7	-4.7
Height	9	CS Apr 09	-4.4	0.4
Height	9	CS Apr 09	-4.1	1.5
Height	9	CS 10	-7.7	9.6
Height	9	CS 10	-8.	-0.3
Height	9	WE 10	-4.2	2.7
Height	9	WE 10	-4.3	2.9
Height	9	BLUP	-4.7	2.2
Height	9	BLUP	-4.9	2.3
Height	9	BLUP	-3.8	0
Exertion	1	CS Apr 09	1.1	-1
Exertion	1	BLUP	0.3	-0.4
Exertion	1	BLUP	0.2	-0.3
Exertion	4	CS Apr 09	1.1	-0.4
Exertion	4	CS Apr 09	1.	-0.2
Exertion	4	CS Apr 09	1.1	-0.3
Exertion	4	BLUP	0.3	-0.2
Exertion	4	BLUP	0.3	-0.2
Exertion	6	WE 10	-0.4	-0.6
Exertion	7	BLUP	0.3	0.1
Exertion	8	CS 10	0.7	-0.7
Exertion	9	WE 10	-0.7	0
Flowering time	1	CS Apr 09	-1.6	2.3
Flowering time	4	CS Apr 09	-2.2	1.1
Flowering time	4	CS Jun 09	-2	1.5
Flowering time	4	CS Jun 09	2.1	-1.3
Flowering time	4	BLUP	-0.3	0.1
Flowering time	6	CS Apr 09	-1.8	0.5
Flowering time	6	CS Jun 09	-2.4	1.5
Flowering time	6	BLUP	-0.3	0.1
Flowering time	10	CS Apr 09	-1.4	1.5
Brix	1	CS Apr 09	0.2	-1.5
Brix	1	CS 10	-1	0.1
Brix	1	CS 10	-1	0.4
Brix	1	BLUP	-0.2	0
Brix	2	CS Apr 09	-0.5	0.4
Brix	2	CS Jun 09	-0.4	0
Brix	2	BLUP	-0.2	0
Brix	3	CS Apr 09	-0.8	-0.1
Brix	3	CS 10	-0.8	0.3
Brix	3	BLUP	-0.3	0
Brix	3	BLUP	-0.3	0.1
Brix	4	WE 10	0.2	0.6
Brix	4	BLUP	0.2	0.1
Brix	6	CS 10	-0.1	-1.3
Brix	9	CS Jun 09	-0.5	0.1
Brix	9	CS Jun 09	-0.5	0.2
Brix	10	CS Jun 09	-0.4	0.8
Harvest yield	3	WE 10	0.4	-0.5
Harvest yield	3	WE 10	0.3	0.4
Harvest yield	3	BLUP	0.2	-0.1
Harvest yield	4	BLUP	-0.2	0.2
Harvest yield	7	CS Jun 09	-0.5	0.7
Harvest yield	7	CS Jun 09	-0.3	0.5
Harvest yield	9	CS 10	-0.5	0.1
Harvest yield	9	BLUP	-0.3	0.1
Vegetative yield	3	CS 10	0.4	-0.3
Vegetative yield	3	WE 10	0.2	0.6
Vegetative yield	3	BLUP	0.2	-0.1
Vegetative yield	4	BLUP	-0.2	0.2
Vegetative yield	7	CS Jun 09	-0.5	0.6
Vegetative yield	7	CS Jun 09	-0.3	0.5
Vegetative yield	9	CS 10	-0.5	0.1
Vegetative yield	9	BLUP	-0.3	0.1
Panicle yield	8	WE 10	-0.1	0
Panicle yield	8	BLUP	0	0
Percent moisture A	7	CS Jun 09	0	-2.7
Percent moisture A	7	BLUP	-0.1	0
Percent moisture B	1	CS Apr 09	-0.4	2.6
Percent moisture B	1	CS Apr 09	-2.1	1.6
Percent moisture B	4	BLUP	-0.2	0.2
Percent moisture B	5	CS Apr 09	-0.9	-0.1
Juice yield	4	WE 10	-141.6	40.6
Juice yield	7	CS Jun 09	-20	226.6
Juice yield	7	CS Jun 09	-121.6	1724
Juice yield	7	CS Jun 09	-130.2	213.9
Juice yield	9	BLUP	-61.1	31.3
Juice yield	9	BLUP	-63.6	40.9

Table 4. Pearson correlation coefficients of phenotypic traits measured in all environments.

Trait	Exertion	Flowering time	Lodging	Harvest yield	Vegetative yield	Panicle yield	Percent moisture A	Percent moisture B
Height	0.05	0.22***	0.05	0.48***	0.52***	-0.13***	0	0.13***
Exertion	--	0.32***	-0.18***	-0.08**	-0.12***	0.29***	0.40***	0.26***
Flowering time		--	-0.4***	0.39***	0.34***	0.45***	0.31***	0.19***
Lodging			--	-0.1***	-0.08**	-0.17***	-0.15***	-0.07*
Harvest yield				--	0.99***	0.46***	0.16***	-0.03
Vegetative yield					--	0.34***	0.1***	-0.05
Panicle yield						--	0.46***	0.15***
Percent moisture A							--	0.38***

Table 4. Continued.

Trait	Dry biomass A	Dry biomass B	Juice yield	Juice by height	Brix	Sugar yield	Extraction efficiency	Cellulose
Height	0.52***	0.37***	0.39***	-0.01	0.10***	0.39***	-0.03	-0.1***
Exertion	-0.21***	-0.22***	0.12***	0.1***	-0.1***	0.07*	0.37***	0.23***
Flowering time	0.26***	0.22***	0.42***	0.32***	0.15***	0.40***	0.29***	-0.19***
Lodging	-0.05	-0.06	-0.15***	-0.19***	-0.09**	-0.16***	-0.15***	0.07*
Harvest yield	0.95***	0.89***	0.86***	0.69***	0.29***	0.86***	0.17***	-0.17***
Vegetative yield	0.97***	0.90***	0.84***	0.64***	0.28***	0.83***	0.12***	-0.19***
Panicle yield	0.23***	0.27***	0.5***	0.55***	0.16***	0.48***	0.45***	0.11***
Percent moisture A	-0.13***	-0.05*	0.6***	0.66***	-0.07**	0.5***	0.98***	0.28***
Percent moisture B	-0.14***	-0.45***	0.16***	0.12***	-0.45***	-0.01	0.18***	0.21***
Dry biomass A	--	0.91***	0.68***	0.46***	0.30***	0.71***	-0.11***	-0.26***
Dry biomass B		--	0.67***	0.51***	0.43***	0.74***	0.05	-0.26***
Juice yield			--	0.9***	0.18***	0.94***	0.56***	0.01
Juice by height				--	0.01	0.83***	0.68***	0.14***
Brix						0.47***	0.03	-0.47***
Sugar yield						--	0.54***	-0.14***
Extraction efficiency							--	0.26***

Table 4. Continued.

Trait	Lignin	Arabinan	Galactan	Xylan	Protein	Ash	Glucan	Sucrose
Height	-0.03	-0.07*	-0.27***	-0.21***	-0.42***	-0.47***	0.21***	0.06*
Exertion	0.27***	0.15***	0.06	0.32***	-0.09**	-0.05	0.18***	-0.17***
Flowering time	0.04	-0.08**	-0.17***	0.02	-0.37***	-0.17***	0.48***	-0.06
Lodging	-0.02	-0.04	0.03	-0.02	0.08**	0.03	-0.16***	0.03
Harvest yield	-0.15***	-0.29***	-0.24***	-0.22***	-0.19***	-0.2***	0.21***	0.19***
Vegetative yield	-0.17***	-0.29***	-0.24***	-0.25***	-0.2***	-0.21***	0.2***	0.2***
Panicle yield	0.09***	-0.1***	-0.05	0.16***	0.02	0.01	0.17***	0
Percent moisture A	0.28***	0.04*	0.12***	0.31***	-0.13***	-0.07**	0.29***	-0.14***
Percent moisture B	0.42***	0.46***	0.11***	0.39***	-0.11***	-0.05	0.08*	-0.49***
Dry biomass A	-0.24***	-0.3***	-0.27***	-0.33***	-0.17***	-0.19***	0.13***	0.24***
Dry biomass B	-0.32***	-0.45***	-0.25***	-0.37***	-0.11***	-0.13***	0.13***	0.38***
Juice yield	0.01	-0.2***	-0.13***	-0.03	-0.23***	-0.2***	0.3***	0.08**
Juice by height	0.13***	-0.1***	0.08**	0.15***	-0.06*	0	0.21***	-0.02
Brix	-0.61***	-0.57***	-0.42***	-0.59***	0.01	-0.1***	-0.05	0.64***
Sugar yield	-0.17***	-0.35***	-0.23***	-0.2***	-0.19***	-0.19***	0.25***	0.27***
Extraction efficiency	0.2***	-0.05	0.09	0.25***	-0.12***	-0.08**	0.3***	-0.05
Cellulose	0.69***	0.50***	0.32***	0.83***	0.23***	0.21***	0	-0.56***
Lignin	--	0.8***	0.54***	0.92***	0	0.01	0.29***	-0.89***
Arabinan		--	0.56***	0.75***	0.1***	0.12***	0.01	-0.85***
Galactan			--	0.56***	0.32***	0.43***	0.01***	-0.5***
Xylan				--	0.22***	0.28***	0.1***	-0.83***
Protein					--	0.84***	-0.65***	-0.09**
Ash						--	-0.48***	-0.21***
Glucan							--	-0.2***

Table 5. QTL locations, LOD scores, and intervals for traits using RIL CIM analysis method.

Trait	Chrom	Location	QTL Peak (cM)	QTL Interval (cM) †	LOD Score	Effect	R^2
Height	7	CS Apr 09	52.9	52.5-54.3	3.4	-2.67	0.07
Height	7	CS Apr 09	63.3	58.5-63.8	7.5	-4.06	0.15
Height	9	CS Apr 09	62.7	57.1-71	4.1	-2.96	0.08
Height	9	CS 10	64.7	59.3-71	5.3	-5.91	0.12
Height	4	WE 10	2	0-5.4	4.3	-2.51	0.09
Height	4	WE 10	9.4	5.4-18.5	4.3	-2.59	0.10
Height	9	WE 10	62.7	51.5-65	2.8	-2.14	0.06
Height	4	BLUP	7.4	4.9-10.3	3.2	-1.71	0.07
Height	4	BLUP	14.3	10.3-30.4	3.3	-1.64	0.07
Height	7	BLUP	58.6	54.3-67.8	3.0	-1.57	0.05
Height	9	BLUP	62.7	60-65	7.2	-2.69	0.15
Exertion	1	CS Apr 09	69.4	65.6-75.4	3.9	0.67	0.08
Exertion	3	CS Apr 09	51.4	39.5-52	3.3	0.56	0.06
Exertion	3	CS Apr 09	58.8	52-63.9	3.2	0.57	0.07
Exertion	6	WE 10	0	0-5.5	5.2	-0.47	0.10
Exertion	8	WE 10	17.2	8.4-29.5	3.5	-0.39	0.07
Exertion	9	WE 10	37.6	20.7-47.3	4.6	-0.52	0.13
Exertion	10	WE 10	8.6	2.1-22.9	4.2	0.44	0.09
Exertion	4	BLUP	61.1	56.8-71.9	3.1	0.17	0.06
Exertion	7	BLUP	83	72.2-95.5	4.0	0.30	0.15
Flowering_time	4	CS Apr 09	9.4	5.4-14.6	5.7	-1.63	0.13
Flowering_time	6	CS Apr 09	0	35.2-39.5	5.5	-1.97	0.12
Flowering_time	4	CS Jun 09	0.1	0-9.7	4.1	-1.48	0.08
Flowering_time	6	CS Jun 09	0	35.2-41.9	4.6	-1.54	0.09
Flowering_time	9	CS Jun 09	71	63.4-73	5.1	-1.81	0.12
Flowering_time	4	BLUP	2	0-12.5	5.1	-0.19	0.11
Flowering_time	6	BLUP	0	35.2-39.5	6.5	-0.21	0.13
Lodging	3	CS Apr 09	93.2	81.7-104.1	4.1	0.46	0.10
Lodging	7	CS Apr 09	12.3	9.2-19	3.0	-0.51	0.07
Lodging	1	WE 10	17.8	6.724.9	3.1	-0.22	0.07
Harvest_yield	3	CS Apr 09	80	69.8-92.5	3.7	0.29	0.08
Harvest_yield	7	CS Jun 09	56.1	54.3-62.7	4.3	-0.27	0.09
Harvest_yield	8	CS Jun 09	63.5	54.9-68.9	5.0	-0.30	0.12
Harvest_yield	9	CS Jun 09	65	61.4-71	3.0	-0.24	0.07
Harvest_yield	3	WE 10	76.2	67.8-81.7	4.5	0.37	0.11
Harvest_yield	3	WE 10	144.9	133.4-148.1	3.0	0.27	0.06
Harvest_yield	4	WE 10	2	0-5.4	4.1	-0.31	0.09
Harvest_yield	3	BLUP	78.2	71-90	7.2	0.23	0.17
Harvest_yield	4	BLUP	4	0-14.6	3.5	-0.15	0.07
Harvest_yield	7	BLUP	92.7	70.8-103.5	3.0	-0.17	0.06
Harvest_yield	9	BLUP	69	65-71	3.7	-0.17	0.09
Vegeative_yield	3	CS Apr 09	80	68.8-92.5	3.3	0.26	0.07
Vegeative_yield	7	CS Jun 09	56.1	54.3-63.3	4.1	-0.24	0.08
Vegeative_yield	8	CS Jun 09	63.5	54.2-74	4.3	-0.26	0.10
Vegeative_yield	9	CS Jun 09	64.7	61.4-71	2.9	-0.22	0.07
Vegeative_yield	9	CS 10	62.7	55.5-65	3.5	-0.37	0.07
Vegeative_yield	3	WE 10	76.2	67.6-81.7	4.1	0.34	0.11
Vegeative_yield	3	BLUP	78.2	70.7-87.5	7.4	0.24	0.18
Vegeative_yield	4	BLUP	2	0-10.8	5.9	-0.19	0.12
Vegeative_yield	7	BLUP	94.7	74.9-108	3.7	-0.19	0.08
Vegeative_yield	9	BLUP	71	62.3-73	3.9	-0.19	0.11

Table 5. Continued.

Trait	Chrom	Location	QTL Peak (cM)	QTL Interval (cM) †	LOD Score	Effect	R^2
Panicle_yield	1	CS Apr 09	13.7	12.3-15.8	2.9	0.03	0.07
Panicle_yield	3	CS Jun 09	125.2	114.6-127.5	2.9	-0.02	0.06
Panicle_yield	8	WE 10	87.4	77.5-91.4	5.0	-0.07	0.15
Panicle_yield	3	BLUP	90.5	81.7-96.6	5.8	0.01	0.14
Percent_moisture_A	3	CS Jun 09	97.7	82.9-107.5	3.4	-1.19	0.08
Percent_moisture_B	1	CS Jun 09	179.7	158.8-181.4	4.6	-0.72	0.10
Percent_moisture_B	2	CS Jun 09	101	97.1-108.1	3.6	0.62	0.07
Percent_moisture_B	2	BLUP	23.3	8.2-34.2	3.2	0.23	0.07
Dry_biomass_A	3	CS Apr 09	80	68.1-92.5	3.6	205.90	0.08
Dry_biomass_A	7	CS Jun 09	56.1	54.3-63.3	5.2	-190.72	0.11
Dry_biomass_A	8	CS Jun 09	65.5	53.1-78.3	3.0	-151.50	0.07
Dry_biomass_A	3	CS 10	83.7	69.1-92.6	4.0	294.63	0.09
Dry_biomass_A	9	CS 10	62.7	57.8-65	4.8	-323.34	0.10
Dry_biomass_A	3	WE 10	76.2	67.6-92.6	3.5	227.01	0.09
Dry_biomass_A	3	WE 10	142.9	133.1-148.1	3.3	187.86	0.07
Dry_biomass_A	4	WE 10	2	0-5.4	4.3	-220.53	0.09
Dry_biomass_A	3	BLUP	78.2	69.5-87.7	7.1	164.27	0.16
Dry_biomass_A	4	BLUP	2	0-9.5	6.2	-142.85	0.12
Dry_biomass_A	7	BLUP	96.7	90.6-108.4	3.6	-125.67	0.08
Dry_biomass_A	9	BLUP	71	62.3-73	4.2	-135.90	0.11
Dry_biomass_B	10	CS Apr 09	64.8	56.3-76.5	3.1	-79.95	0.06
Dry_biomass_B	7	CS Jun 09	56.1	54.3-63.8	4.9	-69.83	0.10
Dry_biomass_B	9	CS Jun 09	67	61.4-71	3.5	-67.69	0.09
Dry_biomass_B	1	CS 10	66.7	60.7-73	2.8	-106.65	0.07
Dry_biomass_B	10	CS 10	22.6	9.9-40.7	3.4	116.82	0.09
Dry_biomass_B	3	WE 10	146.9	136.1-148.1	4.5	79.18	0.10
Dry_biomass_B	4	WE 10	2	0-5.4	6.2	-90.08	0.13
Dry_biomass_B	3	BLUP	76.2	71.8-92.5	3.6	43.95	0.08
Dry_biomass_B	4	BLUP	4	0-9.6	5.3	-49.69	0.11
Juice_yield	7	CS Apr 09	58.1	54.2-62.9	4.1	-128.99	0.10
Juice_yield	8	CS Jun 09	65.5	59.5-68.9	3.7	-81.38	0.08
Juice_yield	8	CS Jun 09	70.9	68.9-78.6	3.5	-78.40	0.08
Juice_yield	3	CS 10	62.1	56-66.3	3.9	108.99	0.08
Juice_yield	3	WE 10	87.7	80-92.8	3.8	112.49	0.09
Juice_yield	4	WE 10	4	0-5.4	4.4	-119.26	0.11
Juice_yield	4	WE 10	9.4	5.4-34	4.6	-119.17	0.11
Juice_yield	3	BLUP	78.2	76.3-91.6	4.6	49.83	0.11
Juice_by_height	3	CS 10	85.7	71-95.7	3.8	1.03	0.10
Juice_by_height	3	CS 10	127.3	121.7-138.9	5.2	-1.07	0.11
Juice_by_height	3	WE 10	87.7	80-92.9	3.7	1.21	0.10
Juice_by_height	3	BLUP	78.2	77.1-81.7	4.9	0.51	0.12
Juice_by_height	6	BLUP	45.1	75.6-99	3.3	0.42	0.07

Table 5. Continued.

Trait	Chrom	Location	QTL Peak (cM)	QTL Interval (cM) †	LOD Score	Effect	R^2
Brix	3	CS Apr 09	59.4	57.4-62.1	6.5	-0.61	0.15
Brix	3	CS Apr 09	72.2	64-78.4	8.9	-0.72	0.21
Brix	4	CS Apr 09	49.6	35.2-52.5	3.4	0.39	0.06
Brix	2	CS Jun 09	124	110.7-126.2	3.0	-0.30	0.06
Brix	3	CS Jun 09	72.2	66.3-78.2	3.7	-0.39	0.10
Brix	9	CS Jun 09	62.7	57.1-71	4.4	-0.41	0.10
Brix	1	CS 10	66.7	63.2-69.9	5.9	-0.83	0.12
Brix	1	CS 10	73.6	69.9-76.3	4.7	-0.74	0.10
Brix	3	CS 10	63.8	54.2-66.3	3.5	-0.57	0.07
Brix	3	CS 10	70.2	66.3-79.2	3.6	-0.62	0.08
Brix	3	WE 10	78.2	69.3-91.9	5.9	-0.57	0.14
Brix	1	BLUP	66	57.2-69.9	3.3	-0.17	0.06
Brix	3	BLUP	74.2	67.4-78.5	10.1	-0.30	0.25
Brix	4	BLUP	100.1	88.8-111.6	3.3	0.15	0.06
Sugar_yield	7	CS Apr 09	58.1	54.5-63.7	4.5	-22.40	0.11
Sugar_yield	8	CS Jun 09	61.5	59.5-68.9	2.8	-10.15	0.06
Sugar_yield	1	CS 10	47	32.2-56.8	3.2	-16.78	0.07
Sugar_yield	4	WE 10	4	0-5.4	4.7	-17.64	0.12
Sugar_yield	4	WE 10	12.3	5.4-25.1	5.3	-18.87	0.13
Sugar_yield	4	BLUP	4	0-14.9	3.1	-6.37	0.07
Extract._effic.	3	CS Jun 09	97.7	88.5-107.5	3.5	-0.02	0.08
Extract._effic.	1	CS 10	122.2	111.4-122.4	2.7	-0.02	0.07
Cellulose	6	CS Apr 09	12.6	5.1-17.1	3.1	0.36	0.07
Cellulose	3	CS Jun 09	123	116.5-127.5	4.0	0.37	0.10
Cellulose	1	CS 10	68.6	67.8-69.9	4.6	0.66	0.10
Cellulose	3	CS 10	65.8	57.8-78.2	3.7	0.55	0.08
Cellulose	3	WE 10	68.2	58.3-79.2	3.0	0.44	0.07
Cellulose	1	BLUP	66.7	59.6-69.4	4.4	0.17	0.09
Cellulose	2	BLUP	117.2	107.3-128.1	2.9	0.12	0.06
Cellulose	3	BLUP	66.3	62.6-78.2	5.1	0.16	0.10
Cellulose	4	BLUP	101.3	94.3-111.7	3.1	-0.12	0.06
Lignin	3	CS Apr 09	78.2	67.9-87.7	5.4	0.22	0.13
Lignin	3	CS Apr 09	92.5	88.5-97.8	3.8	0.18	0.09
Lignin	7	CS Jun 09	58.1	53.7-63.8	3.9	0.39	0.09
Lignin	9	CS Jun 09	58.3	50.4-65	3.4	0.27	0.07
Lignin	3	BLUP	68.2	64.7-78.3	7.5	0.13	0.16
Lignin	6	BLUP	17.1	6.4-19.1	3.1	0.08	0.06
Lignin	7	BLUP	63.8	60.8-67.8	3.4	0.09	0.07
Arabinan	1	CS Apr 09	28	25.5-46.2	3.5	-0.06	0.07
Arabinan	1	CS Apr 09	134.3	132.9-145.5	3.6	-0.06	0.07
Arabinan	3	CS Apr 09	24.2	11-31.6	3.1	-0.06	0.06
Arabinan	6	CS Apr 09	15.8	11.1-17.1	5.1	0.07	0.10
Arabinan	9	CS Jun 09	69	61.4-71	5.6	0.15	0.16
Arabinan	10	CS 10	57.2	46.8-64	3.3	-0.10	0.07
Arabinan	2	BLUP	17.3	1.6-27.9	3.5	0.04	0.09
Arabinan	4	BLUP	0.1	0-14.8	2.9	0.03	0.07
Arabinan	7	BLUP	85	72.2-90.6	3.4	0.05	0.12

Table 5. Continued.

Trait	Chrom	Location	QTL Peak (cM)	QTL Interval (cM) †	LOD Score	Effect	R^2
Galactan	4	CS Apr 09	86.7	82.6-88.8	4.2	0.01	0.09
Galactan	4	CS Apr 09	94.3	89.8-101.3	6.0	0.01	0.14
Galactan	4	CS Apr 09	103.5	101.3-105.5	4.7	0.01	0.10
Galactan	4	CS Jun 09	80.7	77-82.4	4.2	0.01	0.08
Galactan	4	CS Jun 09	86.7	82.4-93.2	4.7	0.02	0.10
Galactan	4	CS Jun 09	98.1	97.4-100.1	9.6	0.02	0.23
Galactan	4	CS 10	102.7	96.8-106.5	5.3	0.02	0.12
Galactan	4	WE 10	88.8	88.8-92.8	5.3	0.02	0.12
Galactan	4	BLUP	81.8	77.7-86.7	5.2	0.01	0.09
Galactan	4	BLUP	92.8	92.5-93	13.6	0.01	0.32
Galactan	4	BLUP	103.5	100.2-114.1	6.9	0.01	0.12
Xylan	2	CS Apr 09	103	97.1-114.5	3.9	0.18	0.09
Xylan	9	CS Jun 09	58.3	52.3-65	4.0	0.26	0.08
Xylan	9	CS Jun 09	71	65-73	3.8	0.28	0.10
Xylan	1	CS 10	68.6	60.2-78.4	3.3	0.36	0.07
Xylan	5	WE 10	78.1	75.6-84	3.5	0.28	0.08
Xylan	1	BLUP	66.7	66-73.7	4.1	0.11	0.09
Xylan	3	BLUP	68.2	63-78.8	4.1	0.10	0.09
Protein	2	CS Jun 09	6	0-21.9	4.1	0.23	0.13
Protein	4	WE 10	2	0-15	3.0	0.13	0.07
Protein	7	BLUP	81	68.2-92.7	3.5	0.07	0.14
Ash	4	CS Apr 09	82.4	77.6-87.7	3.4	0.12	0.07
Ash	7	CS Apr 09	77	58.5-89.9	3.6	0.17	0.11
Ash	2	BLUP	106.8	97.1-118.3	3.0	0.05	0.06
Ash	4	BLUP	8.4	0-17.7	3.2	0.05	0.08
Ash	4	BLUP	106.5	95.8-114.7	3.7	0.05	0.07
Sucrose	7	CS Apr 09	63.8	53.5-67.8	4.0	-0.57	0.09
Sucrose	9	CS Jun 09	58.4	53.6-61.4	5.2	-0.88	0.11
Sucrose	9	CS Jun 09	69	65-71	4.9	-0.94	0.14
Sucrose	5	WE 10	75.3	70.9-83.1	3.8	-0.96	0.09
Sucrose	3	BLUP	68.2	63.6-77.6	5.3	-0.37	0.11
Sucrose	7	BLUP	63.8	59.8-67.8	5.1	-0.38	0.11
Sucrose	7	BLUP	79	69-89.5	4.2	-0.55	0.19

† QTL interval displayed is the 2-LOD interval.

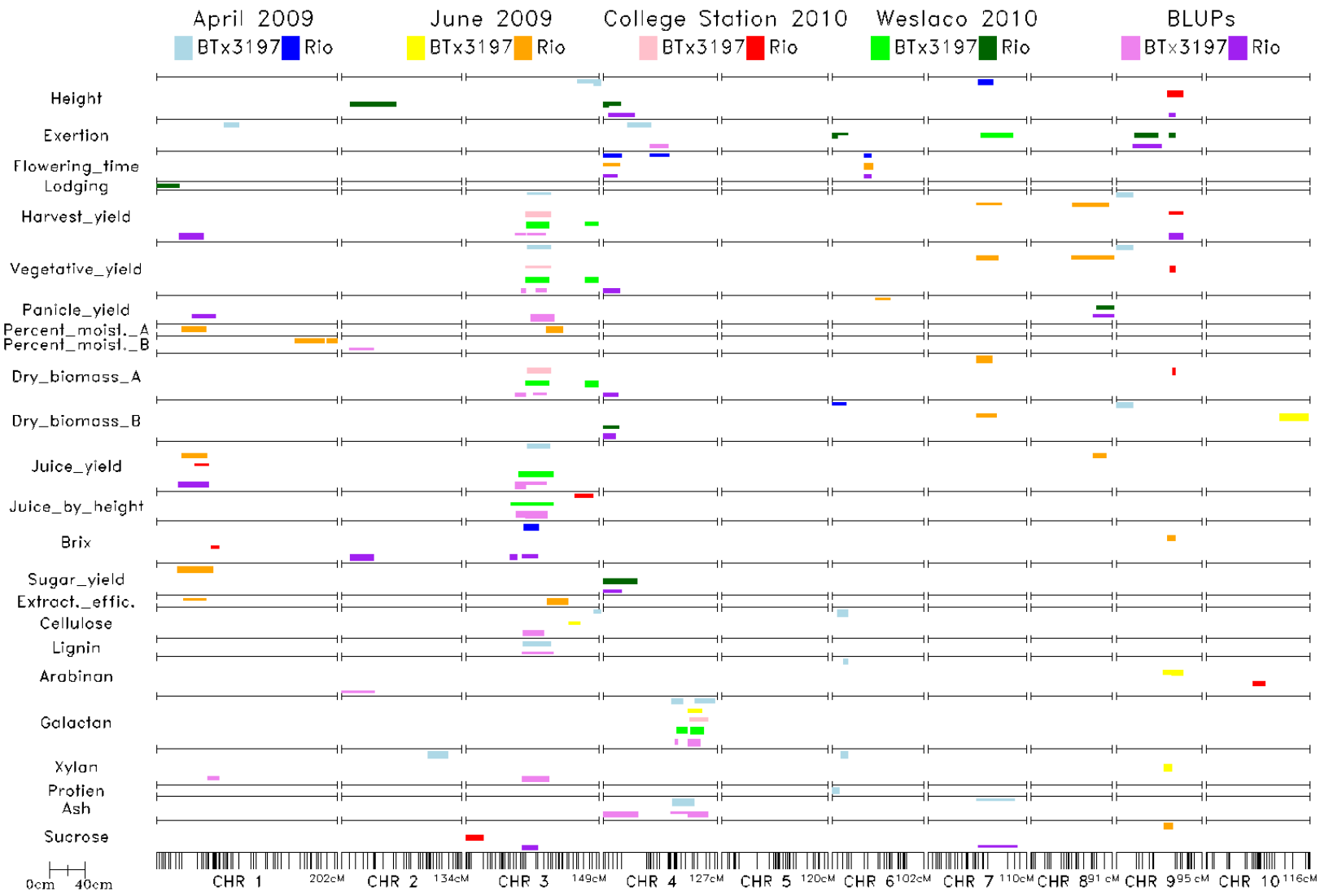

Figure 4. Estimated locations of QTL for traits using an RIL SM analysis method.

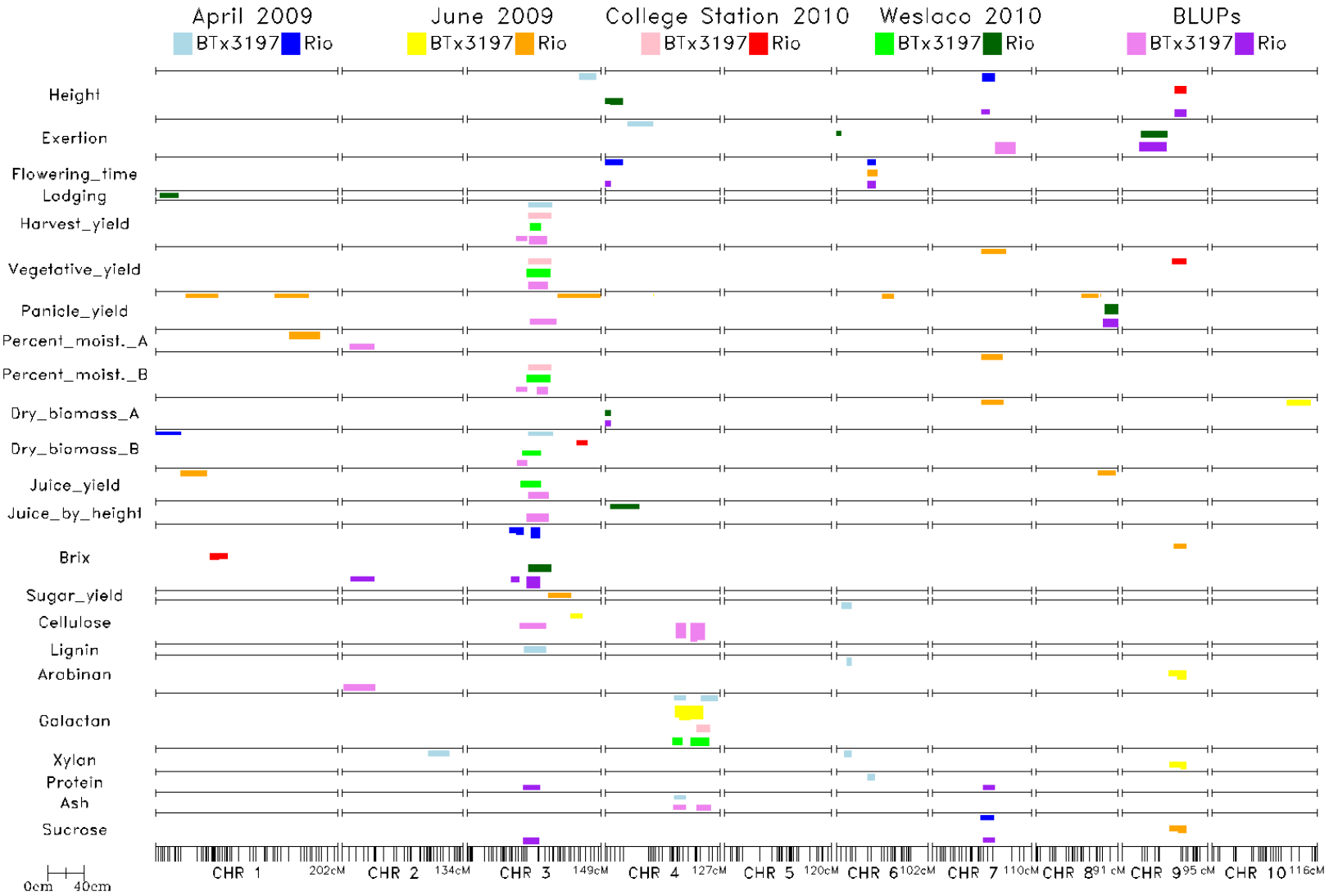

Figure 5. Estimated locations of QTL for traits using an RIL IM analysis method.

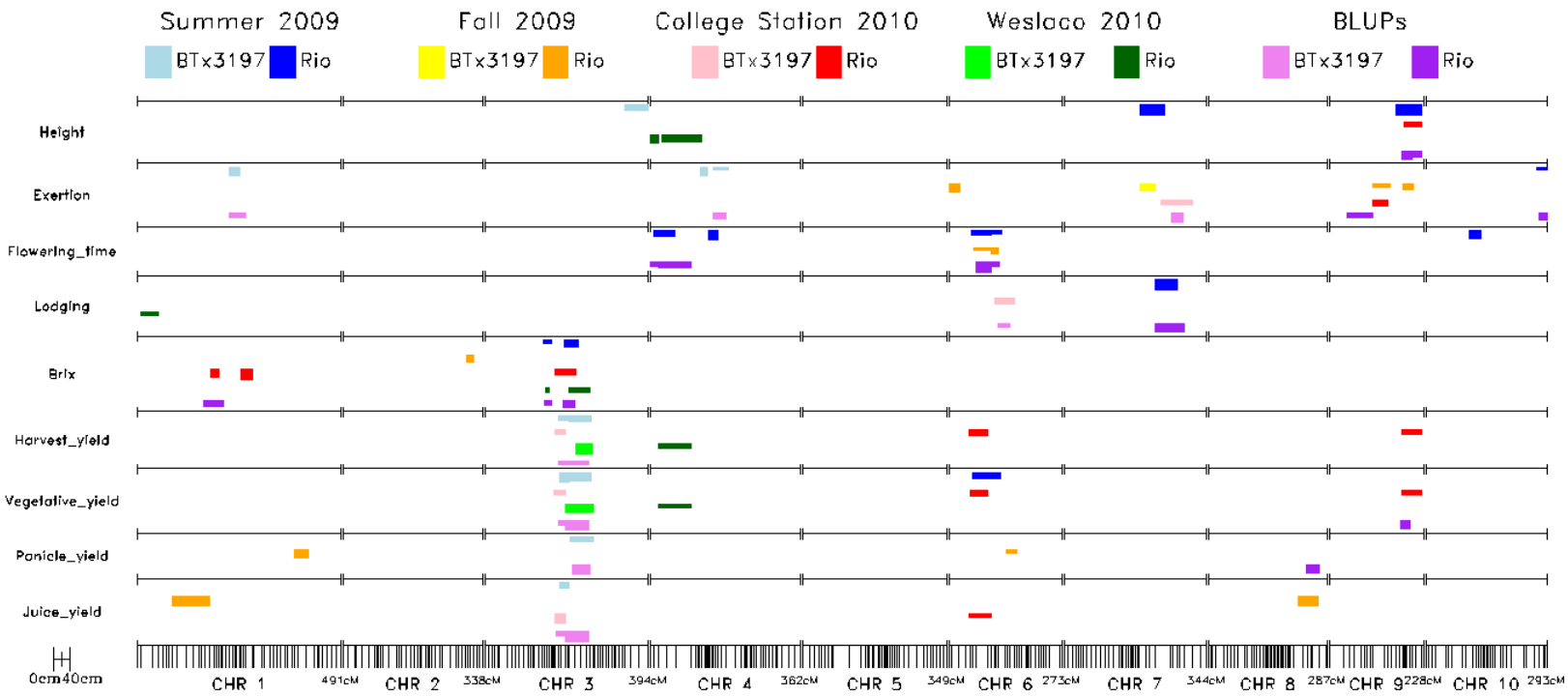

Figure 6. Estimated locations of QTL for traits using an F_3 SM analysis method.

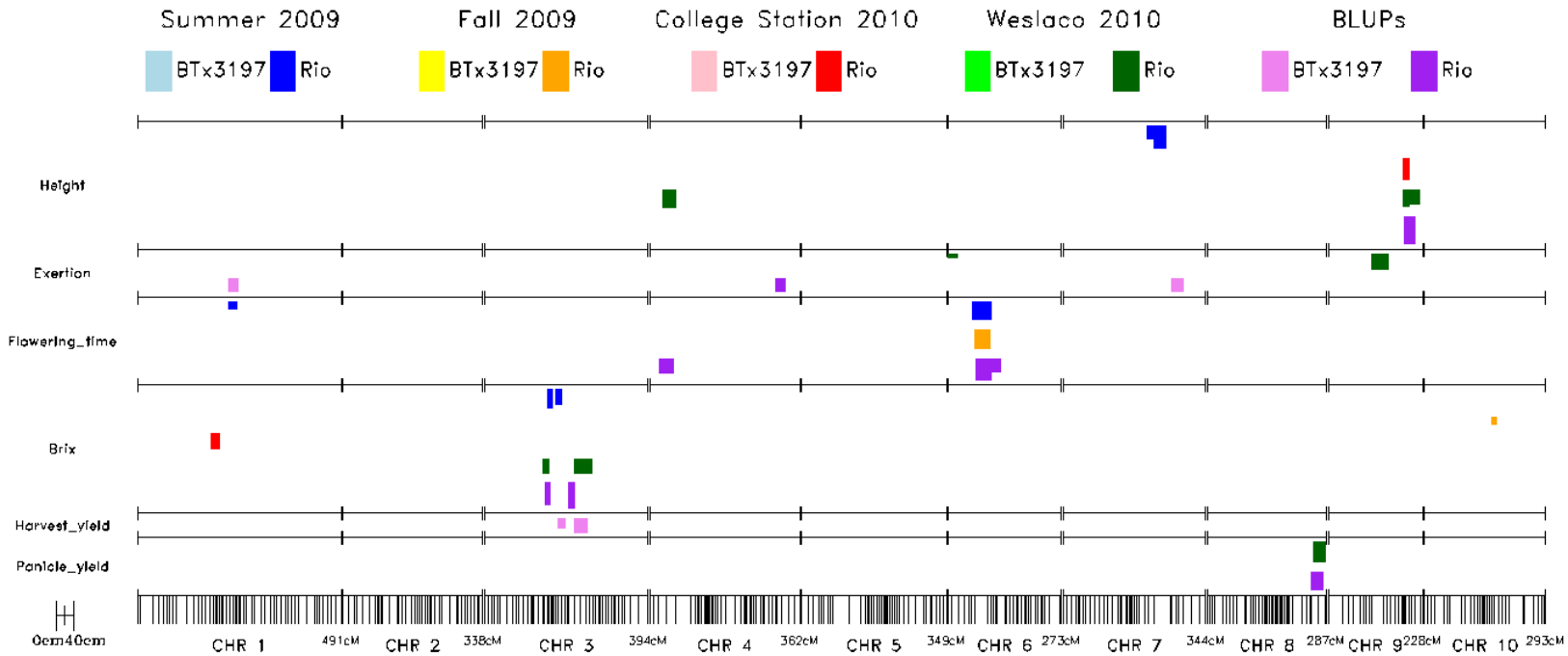

Figure 7. Estimated locations of QTL for traits using an F_3 IM analysis method.

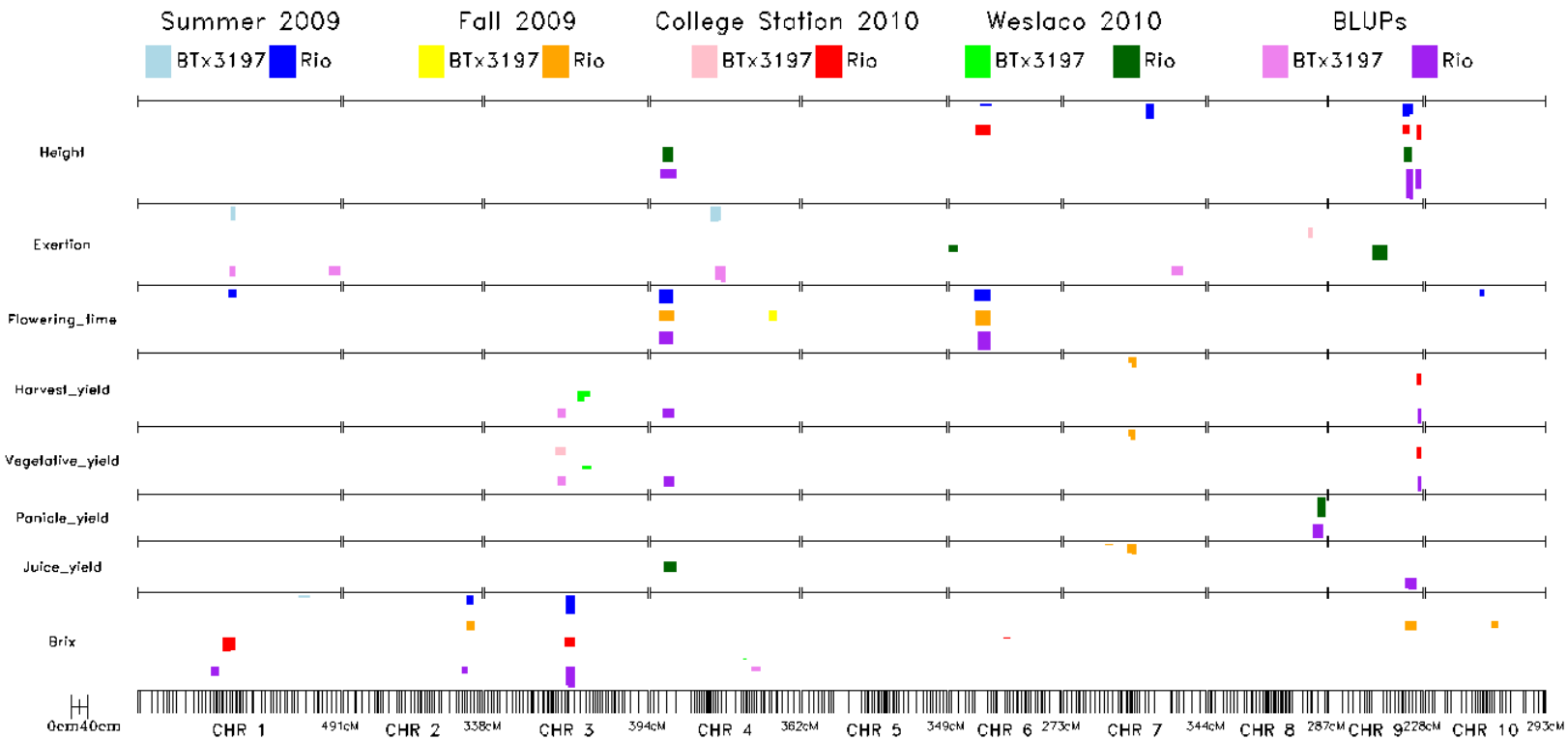

Figure 8. Estimated locations of QTL for traits using an F_3 CIM analysis method.

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