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# QTLs for Biomass and Developmental Traits in Switchgrass (*Panicum virgatum*)

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**Abstract** Genetic and genomic resources have recently been developed for the bioenergy crop switchgrass (*Panicum virgatum*). Despite these advances, little research has been focused on identifying genetic loci involved in natural variation of important bioenergy traits, including biomass. Quantitative trait locus (QTL) mapping is typically used to discover loci that contribute to trait variation. Once identified, QTLs can be used to improve agronomically important traits through marker-assisted selection. In this study, we conducted QTL mapping in Austin, TX, USA, with a full-sib mapping population derived from a cross between tetraploid clones of two major switchgrass cultivars (Alamo-A4 and Kanlow-K5). We observed significant among-genotype variation for the vast majority of growth, morphological, and phenological traits measured on the mapping population. Overall, we discovered 27 significant QTLs across 23 traits. QTLs for biomass production colocalized on linkage group 9b across years, as well as with a major biomass QTL discovered in another recent switchgrass QTL study. The experiment was conducted under a rainout shelter, which allowed us to examine the effects of differential irrigation on trait values. We found very minimal effects of the reduced watering treatment on traits, with no significant effect on biomass production. Overall, the results of our study set the stage for future crop improvement through marker-assisted selection breeding.

**Keywords** Bioenergy · Biomass · Genetics · Linkage map · *Panicum virgatum* · QTL

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## Introduction

Switchgrass (*Panicum virgatum*) is a large $C_4$ perennial grass species native to North America that has recently been the focus of major research efforts due to its potential as a bioenergy feedstock [1, 2]. Currently, biofuel in the USA is primarily derived from corn starch in the form of ethanol [3]. The use of corn as a bioenergy crop has been widely criticized due to the low net energy return, competition with food production, and the environmental degradation, including eutrophication that can result from agricultural runoff [4–6]. To transition domestic biofuel production away from corn-starch-based ethanol, recent efforts have been made to develop the production of cellulosic ethanol and other biofuels from large perennial grasses, such as switchgrass [2]. Biofuels

produced from perennial grasses are projected to have a much higher net energy return, compete less directly with food production, and be more sustainable due to decreased soil degradation and fertilizer inputs [1, 2]. Beyond bioenergy uses, switchgrass has long been planted as a landscaping plant and forage crop, has been used to mitigate soil erosion, and has been important in the restoration of disturbed habitats [2].

Molecular genetic and genomic resources for switchgrass have been developed over the past two decades [7–13]. These advances have, in turn, made the construction of genetic linkage maps for switchgrass possible [8, 14, 15]. Despite the assembly of multiple switchgrass linkage maps, only one study [16] has thus far utilized those maps to link genotype and phenotype through quantitative trait locus (QTL) analysis. QTL mapping is important for the development of switchgrass as a bioenergy feedstock because it can identify loci that can subsequently be used to facilitate the improvement of agronomically important traits through marker-assisted selection [16, 17]. Marker-assisted selection has additional importance for breeding of perennial plants because it allows selection to occur at the seedling stage rather than waiting years for field phenotypic evaluation [16]. The major limitations to genetic mapping in switchgrass are its large genome size, long establishment time, and its polyploid genome. Switchgrass primarily occurs as a tetraploid or octoploid [7, 18]. The original tetraploidization event appears to be the result of an allopolyploid hybridization between different species [19]. Octoploidy is also common in switchgrass and has apparently occurred multiple times independently [20, 21].

Here, we report the results of a QTL mapping study in a full-sib mapping population resulting from a cross between two tetraploid (2n=18) genotypes that were derived from major cultivars of switchgrass, Alamo-A4 and Kanlow-K5. Both Alamo and Kanlow are lowland ecotype cultivars, which are typically found in wet riparian areas of the southern USA. This is in contrast to the upland ecotype that is found in drier habitats [2, 21]. We hereafter refer to progeny of the A4 × K5 cross as the Albany mapping population, due to its origin at the Western Regional Research Center in Albany, CA, USA. The original linkage map for the Albany population was assembled by Okada et al. [14] and demonstrated for the first time clear evidence of disomic inheritance in switchgrass.

The primary goals of our study were to evaluate trait variation in switchgrass and to identify QTLs underlying genetic variation in those traits. We grew the mapping population in Austin, TX, USA, for two seasons (2011 and 2012) and measured a different set of traits each season. In the first season, we documented the growth rate of the Albany population through the assessment of plant height and tiller number at ten time points. Phenological traits and total biomass accumulation were quantified in both seasons. Multiple individual tiller traits (e.g., leaf shape, tiller mass, tiller length, etc.) were also quantified each season as well as other whole-plant traits. We conducted QTL mapping using a combined outbred linkage that was built from raw genotype data originally collected by Okada et al. [14]. Finally, we evaluated the effects of a reduced watering treatment on switchgrass traits, including biomass.

## Methods

### Experimental Design

The experiment was conducted in a rainout shelter facility (N 30.2845, W −97.7809) located within the 33-ha Brackenridge Field Laboratory property of the University of Texas, in Austin, TX, USA, adjacent to the Colorado River. The rainout shelter has a steel frame (Windjammer Cold Frame, International Greenhouse Company, Danville, IL, USA) of dimensions 18.3×73 m. The shelter was covered with a clear 240-μm polyethylene roof, which reduced photosynthetically active radiation by ~10 %. The walls (2.1 m) and eaves (4.2 m) of the shelter were not covered, so as to allow airflow with the ambient environment. The site elevation is 133 m above sea level. Mean maximum temperature (July–August) is ~35.0 °C, and the mean minimum temperature (December) is ~3.0 °C. Soils are Yazoo sandy loam and are greater than 1.2 m deep.

Each member of the Albany population was clonally divided in Austin, TX, USA, in the summer of 2010. We planted two replicates of 192 genotypes from the mapping population in the third week of October of 2010. Plants were arranged in rows, with 0.9-m spacing between plant centers within rows and 2.1-m spacing between rows. Twelve genotypes from the mapping population were planted in each of the 32 rows. One complete set of Albany genotypes was randomized across even rows, while the other set was randomized across odd rows.

To prevent the spread of applied irrigation water and roots between rows, we inserted 3.2-mm-thick hollow plastic sheets (Regal Plastics, Austin, TX, USA) 1.2 m deep between each row. To minimize edge effects, we planted a border of switchgrass plants around the entire field. Plants were hand-watered with a hose twice a week until late November 2010, at which point plants were only watered once a week. Hand watering continued weekly until the completion of the irrigation system on March 3, 2011. All subsequent irrigation was supplied with an irrigation system that consisted of three parallel strands of 10-mm-diameter irrigation drip tape (T-Tape, John Deere, Moline, IL, USA) separated by 0.4 m running the length of each row. The drip tape had a flow rate of 4.22 L per minute per 100 m of tape. Pressure regulators maintained water pressure in the irrigation system at 0.7 bar. For the remainder of 2011 and until May 31, 2012, all planting rows were irrigated at a rate equal to 90 % of expected plant water requirements

(WRs). WR was calculated on a monthly basis using historical reference evapotranspiration (ETo) data from the TexasET network (http://texaset.tamu.edu). To determine WR, monthly ETo data for the Austin area were multiplied by a crop coefficient (*Kc*); $WR=ETo\times Kc$. The *Kc* (0.9) utilized for the duration of this experiment was obtained from Sudan grass at peak production, as it was the most similar crop to *P. virgatum* on the TexasET network crop coefficient database (http://texaset.tamu.edu/cropcoe.php).

To test the effects of soil water availability on biomass and other traits during the summer months, we conducted a differential irrigation treatment in 2012. For the treatment, one set of clonal replicates (clonal replicates in even rows) received reduced irrigation with respect to the second set of clonal replicates (clonal replicates in odd rows). Differential irrigation began on June 1, 2012 and continued until September 31, 2012, with no further irrigation (as a harvest aid) until harvest on October 24, 2012. The irrigation differential applied was based on the calculated WR, with dry rows receiving 50 % less irrigation than the wet rows. For the first 6 weeks of the treatment period, wet and dry rows were irrigated at 80 and 40 % of WR, respectively. Beginning on July 15, 2012, the treatment levels were reduced to 50 and 25 % of WR to increase the magnitude of the treatment effect. The differences in applied irrigation can be seen in Fig. S1.

## Phenotypic Variation

We quantified multiple phenological traits over the course of both seasons. The dates when traits were measured are listed in Table 1. Each season, we monitored emergence (greening up time) at multiple time points from early February into the spring. We measured the number of days until anthesis (the first open floret on plant) in 2011 and the date of heading (50 % of tillers heading) in 2012. To assess the timing at which plants grew the most during the season, we measured plant height (cm) and tiller number at ten time points each over the course of 2011. We also measured plant height for three times in 2012 to compare growth between years.

In both seasons, we selected three tillers from each plant to quantify a suite of morphological traits. We measured a different set of tiller traits each season. In June 2011, we measured morphological traits on the three tallest tillers of each plant and calculated the mean of each trait across the three tillers. These traits included the length (cm) and width (mm) of the third fully elongated leaf above the ground, the length (cm) and diameter (mm) of the second internode, and the height of the tiller to the tip of the panicle (cm). In the summer of 2012, we destructively collected three single tillers from each plant for trait measurements. We based the timing of tiller collection on plant maturity, with tillers being harvested after the plant produced panicles on 50 % of its tillers (heading date). Selected single tillers possessed an emerging or flowering panicle and were representative in size of each plant's average tiller diameter and length. We cut tillers below the first node that could be located above the soil surface and transported them immediately to the lab for measurement. For each tiller, we measured its total length (cm), base width (mm), length (cm) of the first internode (cm), and length (cm) and width (mm) of the terminal internode and counted the total number of phytomers per tiller. We also measured the length (cm), width (mm), and leaf area ($cm^2$) of the fourth leaf and flag leaf of each tiller. The means for each trait across the three harvested tillers are reported in Table 1.

Two other leaf traits were quantified in 2011. A SPAD meter (Konica-Minolta SPAD 502, Chiyoda, Tokyo, Japan) was used to approximate the chlorophyll content (unitless) of the highest fully elongated leaf on each tiller using the ratio of transmitted light with wavelengths 940 and 650 nm [22]. Switchgrass varies extensively in leaf color hue, with waxy leaves having a bluer hue than less waxy leaves: We visually scored the color hue of each plant on a five-point scale from 1 being light green to 5 being very blue.

To assess biomass, we harvested all aboveground biomass from each plant with a sickle bar mower (BCS America, Portland, OR, USA) at the end of both seasons (Fig. 1). The plants were dried at 57–65 °C to a constant mass (±1 %) and weighed to determine aboveground biomass production (g). We conducted a final tiller count for the season following the harvest in late December by counting the stumps of cut tillers. Plants deemed too large to easily manipulate into drying ovens were ground with a 15-cm-blade chipper, dried at 57–65 °C to a constant mass (±1 %), and weighed to determine aboveground biomass production.

Other whole-plant traits were measured in both years, including early season crown width and mid-season canopy area in 2011 as well as leaf area index and tiller angle in 2012. Canopy area was measured at 0.5 m above the ground by quantifying two perpendicular diameters and calculating the area, with the assumption that the cross-sectional shape of the canopy is an ellipse. We measured the leaf area index (LAI, $m^2\ m^{-2}$) of each plant on March 22 and April 22 using a Decagon LP-80 Ceptometer (AccuPAR model LP-80, Decagon Devices, Inc., Pullman, WA, USA). LAI was determined by averaging two perpendicular measurements taken through the center of each plant at 10 cm above the soil surface. We scored (1–10 scale) the tillering angle of each plant twice in 2012, with a score of 1 representing completely erect (perpendicular to soil surface) tillers and a score of 10 representing completely procumbent (parallel to soil surface) tillers. Finally, we rated the degree of post-harvest regrowth following the 2012 harvest of each plant on a 0–10 scale, with 0 being no regrowth and 10 being very vigorous regrowth.

We calculated the mean, standard error (SE), and range for all traits (Table 1). To test for significant variation among genotypes, we also conducted one-way ANOVAs for each

**Table 1** Mean and variation of traits measured on the Albany mapping populations

| Trait | Date 2011 | Mean | SE | Range | Date 2012 | Mean | SE | Range |
|---|---|---|---|---|---|---|---|---|
| Anthesis date (Julian day) | Multiple days | 181 | 1.77 | 107–242 | – | – | – | – |
| Base tiller width (mm) | – | – | – | – | Summer 2012 | 5.33 | 0.04 | 3.30–7.34** |
| Biomass (g) | December 7, 2011 | 1014.30 | 25.31 | 147–3549** | October 24, 2012 | 3198.73 | 77.60 | 692–10276** |
| Canopy area ($cm^2$) | June 15, 2011 | 10781.97 | 326.14 | 60.8–40860.8 | – | – | – | – |
| Chlorophyll content (SPAD) | June 15, 2011 | 40.67 | 0.21 | 34.4–50.1* | – | – | – | – |
| Crown width (cm) | March 23, 2011 | 8.85 | 0.20 | 0.64–16.51* | – | – | – | – |
| First internode length (cm) | – | – | – | – | Summer 2012 | 24.33 | 0.20 | 14.13–38*** |
| First node width (mm) | – | – | – | – | Summer 2012 | 6.98 | 0.05 | 4.57–10.53* |
| Flag leaf area ($cm^2$) | – | – | – | – | Summer 2012 | 20.55 | 0.51 | 6.18–61.19 |
| Flag leaf length (cm) | – | – | – | – | Summer 2012 | 38.16 | 0.44 | 19.13–73.16* |
| Flag leaf width (mm) | – | – | – | – | Summer 2012 | 13.08 | 0.10 | 7.67–21* |
| Fourth leaf area ($cm^2$) | – | – | – | – | Summer 2012 | 47.70 | 0.77 | 12.28–93.29*** |
| Fourth leaf length (cm) | – | – | – | – | Summer 2012 | 57.82 | 0.47 | 33.73–84.3*** |
| Fourth leaf width (mm) | – | – | – | – | Summer 2012 | 15.47 | 0.11 | 9.00–23.17*** |
| Green-up time (Julian day) | Multiple days | 63.31 | 0.41 | 43–81*** | Multiple days | 57.26 | 0.25 | 43–75*** |
| Heading date (Julian day) | – | – | – | – | Multiple days | 153.27 | 0.96 | 102–197*** |
| Leaf area index 1 | – | – | – | – | March 22, 2012 | 1.84 | 0.03 | 0.48–5.23*** |
| Leaf area index 2 | – | – | – | – | April 22, 2012 | 2.95 | 0.06 | 0.97–12.73 |
| Leaf color (1–5) | June 15, 2011 | 3.64 | 0.03 | 2–5** | – | – | – | – |
| Mean tiller mass (g) | – | – | – | – | Summer 2012 | 22.38 | 0.34 | 3.13–44.67*** |
| Number of phytomers | – | – | – | – | Summer 2012 | 8.86 | 0.08 | 4–12.33* |
| Number of tillers 1 | March 12, 2011 | 7.59 | 0.26 | 0–27*** | – | – | – | – |
| Number of tillers 2 | March 23, 2011 | 9.60 | 0.27 | 0–27*** | – | – | – | – |
| Number of tillers 3 | April 3, 2011 | 9.80 | 0.26 | 0–27*** | – | – | – | – |
| Number of tillers 4 | April 13, 2011 | 11.92 | 0.32 | 1–38*** | – | – | – | – |
| Number of tillers 5 | April 27, 2011 | 22.80 | 0.56 | 1–61*** | – | – | – | – |
| Number of tillers 6 | May 11, 2011 | 27.88 | 0.66 | 3–79** | – | – | – | – |
| Number of tillers 7 | May 26, 2011 | 36.14 | 0.79 | 4–115** | – | – | – | – |
| Number of tillers 8 | June 29, 2011 | 61.06 | 1.26 | 9–155* | – | – | – | – |
| Number of tillers 9 | August 12, 2011 | 103.07 | 2.43 | 26–371* | – | – | – | – |
| Number of tillers 10 | December 8, 2011 | 121.03 | 2.56 | 32–380* | – | – | – | – |
| Plant height 1 (cm) | March 3, 2011 | 4.60 | 0.28 | 0–29.8*** | – | – | – | – |
| Plant height 2 (cm) | March 12, 2011 | 14.89 | 0.41 | 0–39*** | March 12, 2012 | 62.46 | 0.66 | 23–99*** |
| Plant height 3 (cm) | March 23, 2011 | 40.35 | 0.64 | 0–68.58*** | – | – | – | – |
| Plant height 4 (cm) | April 3, 2011 | 68.05 | 0.74 | 19.05–100.33*** | March 29, 2012 | 118.62 | 0.93 | 69–158* |
| Plant height 5 (cm) | April 13, 2011 | 83.86 | 0.77 | 7.62–121.92*** | – | – | – | – |
| Plant height 6 (cm) | April 27, 2011 | 111.05 | 0.94 | 10.16–153.67* | April 18, 2012 | 157.15 | 1.08 | 70–210* |
| Plant height 7 (cm) | May 11, 2011 | 127.95 | 0.95 | 29–174*** | – | – | – | – |
| Plant height 8 (cm) | May 26, 2011 | 147.40 | 1.01 | 73.5–201*** | – | – | – | – |
| Plant height 9 (cm) | June 29, 2011 | 177.32 | 1.02 | 111–239*** | – | – | – | – |
| Plant height 10 (cm) | August 12, 2011 | 186.27 | 1.25 | 41–272*** | – | – | – | – |
| Post-harvest green up | – | – | – | – | November 19, 2012 | 3.20 | 0.12 | 0–10*** |
| Second internode length (cm) | June 15, 2011 | 13.87 | 0.08 | 8.33–19.00*** | – | – | – | – |
| Second internode width (mm) | June 15, 2011 | 5.85 | 0.03 | 3.87–8.20** | – | – | – | – |
| Terminal internode length (cm) | – | – | – | – | Summer 2012 | 16.69 | 0.26 | 5.73–39.20 |
| Terminal node width (mm) | – | – | – | – | Summer 2012 | 5.58 | 0.05 | 3.04–12.21** |
| Third leaf length (cm) | June 15, 2011 | 55.51 | 0.37 | 26.17–73.50*** | – | – | – | – |
| Third leaf width (mm) | June 15, 2011 | 14.94 | 0.07 | 9.00–19.33*** | – | – | – | – |
| Tiller angle 1 | – | – | – | – | March 23, 2012 | 5.08 | 0.07 | 3–7*** |
| Tiller angle 2 | – | – | – | – | April 30, 2012 | 4.98 | 0.06 | 3–9*** |
| Tiller length (cm) | – | – | – | – | Summer 2012 | 189.22 | 1.35 | 96.1–279.6** |

*Date* date of measurement, *SE* standard error

Range includes asterisks to indicate whether among-genotype variation was significant

*$P<0.05$, **$P<0.01$, *** $P<0.001$

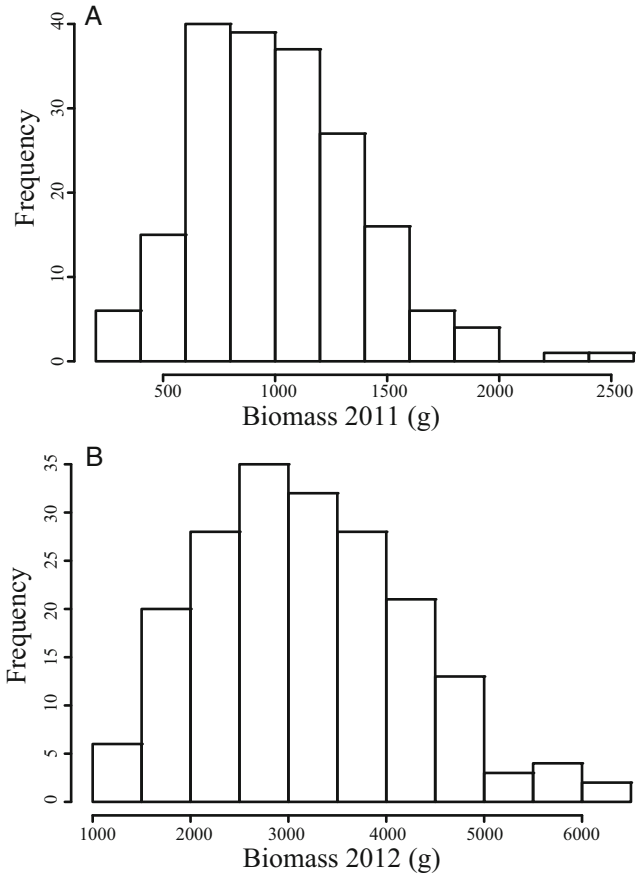



**Fig. 1** Histogram distribution of biomass in 2011 and 2012, with means for Alamo (AP13) and Kanlow (398209) indicated by *squares* and *circles*, respectively

measured trait, except for biomass. Since biomass was quantified in both 2011 and 2012, we analyzed the effects of genotype, year, and the genotype × year interaction on biomass with a two-way ANOVA. All ANOVAs were conducted using the R Language and Environment (version 3.1.2) [23]. Finally, we evaluated the effects of the 2012 watering treatment on traits measured after the initiation of the treatment in June. One-way ANOVAs conducted in R were used to test treatment effects on these traits.

## Growth Modeling

Rates of growth based on plant heights (cm) and tiller numbers, measured during the 2011 growing season, were modeled as continuous functions of time (days) using nonlinear mixed-effects models, implemented in R [23] using the *nlme* package [24]. Random-effects model coefficients were estimated for every plant at the experimental site and used to predict a range of phenotypes. A series of manipulations were necessary to facilitate curve fitting: Both tiller number and growth were log($x$+1)-transformed to improve homogeneity, zero values for tiller number were excluded from the analysis, and the first day on which height measurements were collected (61 Julian days, March 2) was set to $t$=0 (this date was also immediately prior to the first tiller census on which newly emerged tillers were observed).

Tiller number showed a sigmoidal increase during the growing season and was modeled using the four-parameter logistic equation implemented by *SSfpl*:

$$N' = A_N \frac{(B_N - A_N)}{1 + e^{\left(\frac{t_{IP}-t}{\Phi}\right)}}$$

where $N'$ is the transformed number of tillers, $A_N$ is the initial asymptote, $B_N$ is the eventual asymptote, $t$ is the time (days), $t_{IP}$ is the time at which the inflection point of the curve is reached, and $\Phi$ is a scaling coefficient.

Log-transformed plant heights ($H'$) increased asymptotically, so were modeled using the monomolecular function, implemented in R using *SSasymp*:

$$H' = B_H + (H_0 - B_H)e^{-rt}$$

where $H'$ is the transformed plant height, $B_N$ is the eventual asymptotic height, $H_0$ is the height at $t$=0, and $r$ is a rate constant (exp(lrc) in the *nlme* documentation).

Because it was necessary to use a log($x$+1) transformation in order to fit the growth functions, model predictions were back-transformed to the original scale (Fig. 2a, d) prior to the derivation of growth rates. For both tiller number and plant height models, absolute growth rates ($AGR_N$, tillers day$^{-1}$; $AGR_H$, cm day$^{-1}$) were determined as the slopes of the growth functions on the original scale (d$X$/d$t$), using finite differencing. Relative growth rates ($RGR_N$, tillers tiller$^{-1}$ day$^{-1}$; $RGR_H$, cm cm$^{-1}$ day$^{-1}$) were calculated as (d$X$/d$t$)/$X$ [25].

We predicted growth rates and plant sizes from the growth functions (Fig. 2b, c, e, f) and used these predictions to derive several growth traits: the maximum AGR obtained for both tillers and height ($AGR_{N,max}$ and $AGR_{H,max}$), the days on which maximum AGR was obtained for both tillers and height ($t_{AGR,N}$ and $t_{AGR,H}$), and the maximum RGR ($RGR_{N,max}$) and day on which it was achieved ($t_{RGR,N}$) in terms of tillers (we did not calculate equivalent RGR values for plant height because relative growth rates calculated from the monomolecular function decline asymptotically from maximums when $H$>0 that can be extreme as H→0 (Fig. 2b); finally, we predicted the days ($t_{Asym,N}$ and $t_{Asym,H}$) on which tiller number and height were within 5 units (cm, and tillers, respectively) of the predicted asymptotic values on the original scale (exp($B_H$)−1 and exp($B_N$) −1).

## Linkage Map

The first linkage map for the Albany full-sib mapping population was constructed as two separate maps based on recombination events that occurred in the male parent (Alamo-A4) or female parent (Kanlow-K5). To conduct QTL mapping, we first combined the male and female maps together into a single outbred map using the R package OneMap [26]. OneMap is a

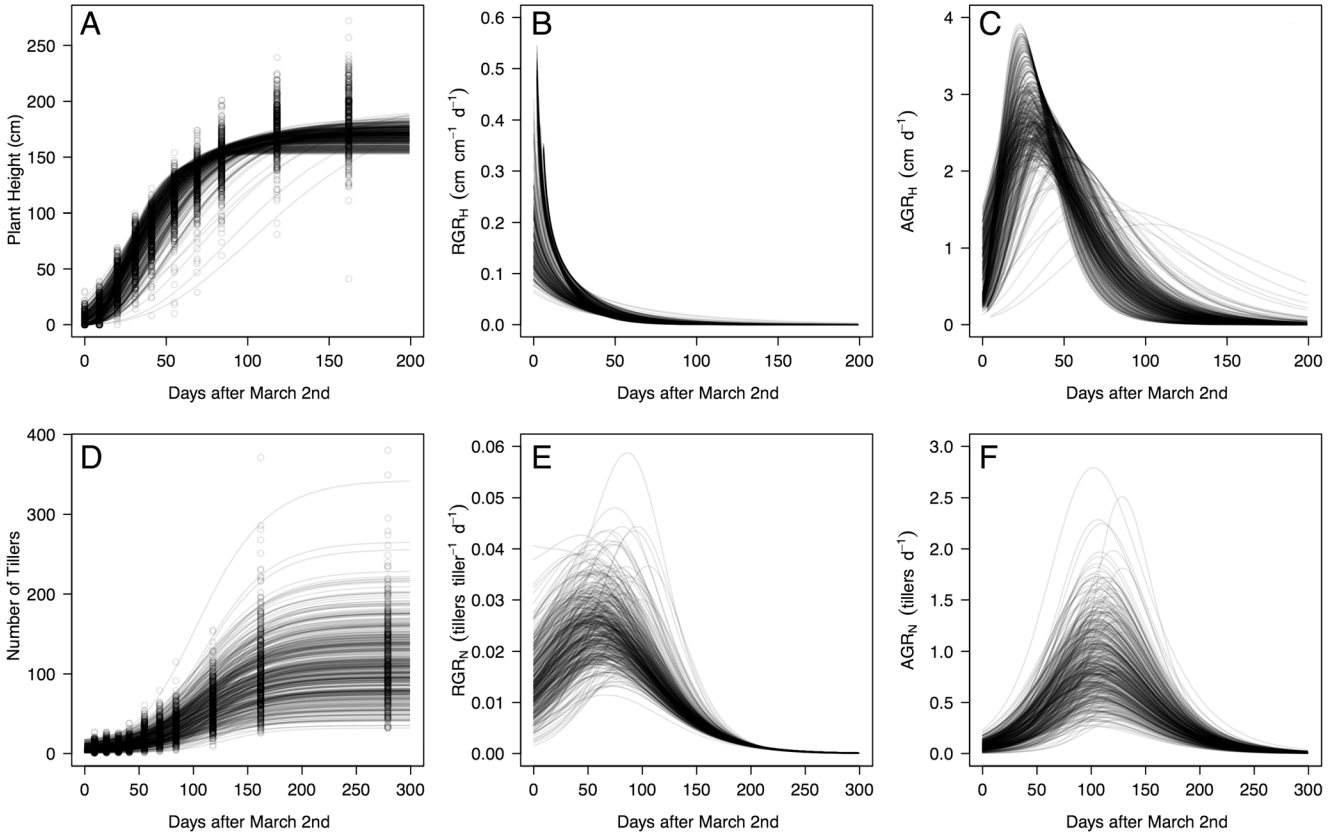



**Fig. 2** Fitted curves for growth of individual plants predicted on a daily time step. Height, fitted as a monomolecular, asymptotic function on a log($x$+1) scale: **a**) plant heights and fitted curves on the original scale, **b** relative growth rates for height (curves have been truncated to eliminate RGR values calculated for $H \leq 1$ cm, which result in unrealistic values), and **c** absolute growth rates for height. Tiller numbers, fitted as a four-parameter logistic function on a log($x$+1) scale after removal of zero counts: **d** tiller numbers and fitted curves on the original scale, **e** relative growth rates for tiller number, and **f** absolute growth rates in terms of tiller accumulation

maximum-likelihood-based methodology for linkage map construction in outbred species, where highly informative markers that are polymorphic in both parents (e.g., AB × CD) are used to unite homologous linkage groups from male and female maps. Okada et al. [14] originally constructed their linkage map by treating all alleles as independent presence/absence markers. In contrast, we evaluated all alleles simultaneously for each marker, which allowed us to group multiple alleles together by genetic locus. Following the grouping of alleles by locus, we determined the segregation category designation of each locus according to the OneMap instructions.

We conducted initial grouping of markers using the two-point algorithm in OneMap (log of odds (LOD)=10, max.rf=0.5). We then constructed each linkage group individually using the following protocol. We used rapid chain delineation (RCD) to identify a preliminary marker order [27] for each linkage group. We then removed all but seven markers for the construction of a framework map. We preferentially selected markers that were the most informative and spread as broadly across the preliminary RCD linkage map as possible. The initial seven markers were ordered with the COMPARE algorithm. We then used the TRY algorithm to place one marker at a time onto the framework map. After each ten additional markers, we checked their order using the RIPPLE algorithm to search for higher likelihood alternative orders within a five-marker window [28]. When adding markers, we tried to avoid adding markers with ambiguous orders, especially when many markers were available.

We began linkage map assembly with 637 markers, which had successfully been assigned OneMap designations and did not have significant segregation distortion at $P<0.001$. Through the process, we strove to make sure that we recovered the full length of the linkage maps assembled by Okada et al. [14], by comparing their map to our early map assemblies. We had difficulty in fully assembling four linkage groups with the set of 637 markers (LG1a, LG1b, LG2b, and LG7b). To complete the assembly of these linkage groups, we searched our pool of markers that had previously been previously filtered for segregation distortion ($P<0.001$) and recovered 41 markers from those four linkage groups. We then added a subset of those markers with the TRY algorithm to the linkage maps to complete their assemblies.

## QTL Mapping

We observed considerable spatial heterogeneity across our field site. Because the trait data showed systematic trends, unrelated to genotype, across the plot, we estimated and

removed these trends prior to QTL mapping. An example of this process can be seen in Fig. S3. Trend estimation and removal were accomplished using the tps from the *fields* package [29] in R [23]. We estimated the trend surface using the tps mixed-effects model where

$$Y_k = Z_k b + P(x_k) + G(x_k) + \epsilon_k$$

where $Y_k$ is the measured trait at location $k$, $Z_k$ is a design matrix that accounts for the covariates when present, $b$ is a vector of coefficients, $P(x_k)$ is a linear spatial trend function at spatial coordinate $x_k$, $G(x_k)$ is a Gaussian random field constructed from radial basis functions, and $\epsilon_k$ are iid. random errors. The degree of smoothing was adjusted using cross validation to minimize

$$g(f) = \int \nabla^2 f(x) dx$$

where $f(x)=P(x)+G(x)$ and $x$ represents the spatial plane. The residuals $\epsilon_k$ are estimates of each trait.

Prior to QTL mapping, we calculated the mean for the two replicates of each clone of Albany population using the spatially adjusted data. We then implemented QTL mapping using R/qtl [30]. We first calculated the probabilities of genotypes using a hidden Markov model (calc.genoprob) under the outbred mapping design. We then conducted genetic mapping using Haley-Knott regression with the scanone function in R/qtl and calculated significance thresholds for each trait with 1000 permutations. The 1.5 LOD drop confidence intervals were calculated for each QTL. We also imputed genotype data with sim.geno and estimated the variance explained and additive effects for each identified QTL with fitqtl. Additive effects were calculated as differences in phenotypic means between individuals with the AC genotype and each of the other three genotypes (AD, BC, and BD) at the QTL. Finally, we conducted mapping of genotype × environment QTLs by first calculating the difference in trait values for each genotype between wet and dry treatments, for traits measured after the initiation of the treatment in June 2012. We then conducted QTL mapping on the values of the difference, using the methods described above, as in Lowry et al. [31].

## Results and Discussion

### Phenotypic Variation

Phenological traits varied greatly among experimental plants. We observed significant among-genotype variation in spring emergence (green-up time) in both 2011 and 2012 (Table 1). We also observed significant among-genotype variation in heading date in 2012. However, there was no significant variation in date of anthesis in 2011. Some whole-plant traits, such as tiller angle, were significantly variable among genotypes, while others, such as canopy area, were not (Table 1). All individual tiller traits varied significantly among genotypes. There was also significant variation among genotypes for leaf color and leaf chlorophyll content (Table 1).

Growth in plant height and tiller production both had a sigmoidal shape in 2011, with slow early season growth, rapid mid-season growth, and a late season asymptote (Fig. 2a). Plant height began its rapid growth phase around March 12, while new tiller production did not reach a rapid growth phase until later in the growing season (Fig. 2d). Plant height began to level off around June 29, while new tiller production leveled off around August 12. Plant height growth occurred much more rapidly in 2012 than in 2011, with plants achieving a mean height of 157.15 cm on April 18, 2012 but only 111.05 cm on April 27, 2011. The more rapid growth in 2012 likely reflects the more moderate winter conditions of that year and the fact that plants were better established in 2012 than in 2011.

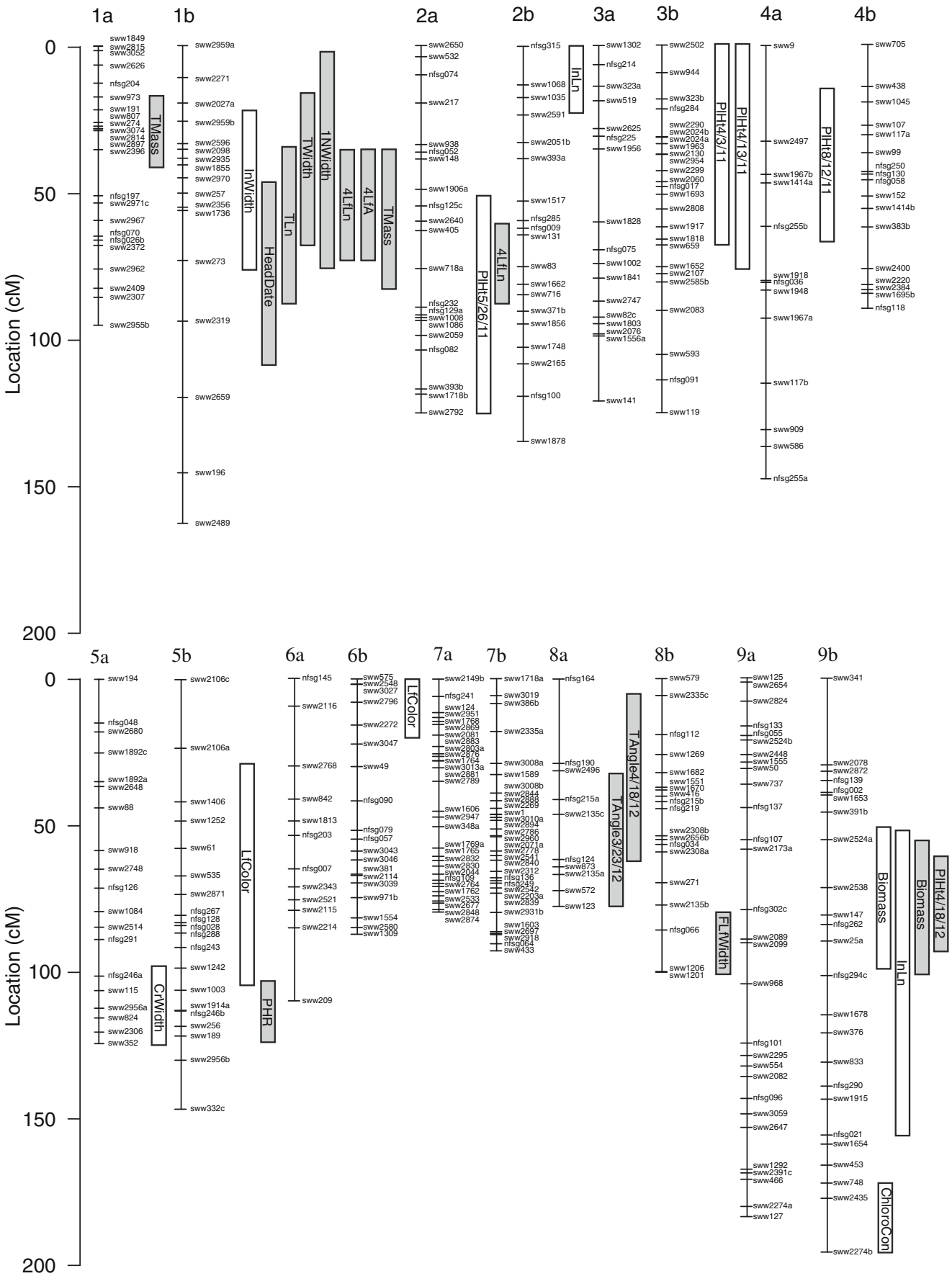
There was a high degree of variation in total biomass production in both 2011 and 2012 (Fig. 1), with some plants over an order of magnitude larger in size than other plants (Table 1). Mean biomass was more than three times greater in 2012 than in 2011 (Tables 1 and 2), which reflects vigorous growth following establishment. There was also significant among-genotype variation in biomass (Table 2).

Only four traits were significantly affected by the watering treatment in 2012. These traits included tiller length ($F_{1,378}$= 9.735, $P$=0.00195), fourth leaf length ($F_{1,378}$=3.888, $P$= 0.0494), fourth leaf area ($F_{1,378}$=4.226, $P$=0.0405), and mean tiller mass ($F_{1,378}$=4.671, $P$=0.0313). Biomass was not significantly affected by the watering treatment ($F_{1,378}$=1.676, $P$=0.196). The lack of effect of the watering treatment on biomass could be the result of the treatment's late initiation in the growing season (June), after which point plants had already accumulated a fair amount of aboveground biomass. Regardless of the cause, these results suggest that watering during summer months can be reduced in central Texas without major consequences on overall yield for the season. Long-term, however, reduced watering of a perennial grass in one season could have consequences for biomass yield in subsequent years. We are currently in the process of studying those legacy effects in other experiments.

**Table 2** Analysis of variance of biomass production in the Albany population

| Sources | Df | Mean squares | *F* value |
|---|---|---|---|
| Genotype | 191 | 1,771,531 | 1.352** |
| Year | 1 | 907,971,795 | 692.870*** |
| Genotype × year | 378 | 669,439 | 0.511 |
| Residuals | 378 | 1,310,450 | |

**$P$<0.01, ***$P$<0.001


1a
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9a
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Location (cM)
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200
TMass
InWidth
HeadDate
TLn
TVWidth
1NWidth
4LfLn
4LfA
TMass
PlHt5/26/11
4LfLn
InLn
PlHt4/3/11
PlHt4/13/11
PlHt8/12/11
LfColor
CrWidth
LfColor
PHR
TAngle4/18/12
TAngle3/23/12
FLfWidth
Biomass
InLn
Biomass
PlHt4/18/12
ChloroCon

## Linkage Map

We successfully assembled a combined outbred linkage map for the full-sib population using OneMap. The final linkage map included 366 markers across 18 linkage groups, with a total map length of 2200.75 cM (Fig. 3). The map had an average intermarker spacing of 6.3 cM, with 3.4 % missing genotype data. There were 100 fully informative, four allele (A, B, C, and D), markers included in the final map, which helped to unite male and female linkage groups together. The recombination fractions of the final map (Fig. S2) generally supported the order and linkage group association of markers. The linkage groups were named by the same conventions used in previous switchgrass linkage mapping studies [14–16].

## QTL Mapping

We conducted QTL mapping on the 56 traits reported in Table 1. Overall, we mapped 27 significant QTLs across 23 of those traits (Fig. 3, Table 3). We detected no significant QTLs for the remaining 33 traits. One of the most significant findings of our study was the identification of a major biomass QTL. We mapped the same QTL for biomass production on linkage group (LG) 9b, centered on marker nfsg262, in both 2011 and 2012. This QTL was primarily caused by the effect of alternative alleles contributed by the Alamo-A4 parent of the hybrid population (Fig. 4). The additive effect of the difference between the two Alamo-A4 alleles accounted for 208 g of biomass per plant (20.5 % of the mean biomass) in 2011 and 565 g per plant (17.7 % of the mean biomass) in 2012 (Fig. 3). The Kanlow-K5 alleles also had an effect on biomass, albeit weaker, accounting for 42 g in 2011 and 252 g in 2012.

Seven of the 27 (25.9 %) QTLs colocalized to one region of LG 1b. All of these colocalizing QTLs had effects on individual tiller traits including tiller width, tiller length, tiller mass, and leaf area (Table 3). The overlap of so many QTLs in this region suggests that selection upon this locus could be useful for changing tiller architecture. There was also a QTL for heading date that had overlapping confidence intervals with the seven tiller QTLs on LG 1b. While we detected this QTL for heading date in 2012, we did not detect any QTLs for anthesis date in 2011.

We mapped QTLs for plant height at four different points during the 2011 season. There were colocalizing QTLs for plant height on LG 3b for two consecutive measurement dates (April 3 and April 13), but QTLs from later in the season mapped to different LGs (LG 2a on May 26 and LG 4a on August 12). We only mapped a single QTL for plant height at one time point in 2012 (April 18), which colocalized with the major biomass QTL on LG 9b. Serba et al. [16] found that some QTLs for end of season plant height were consistently detected across environmental conditions, so there is potential to use marker-assisted selection to stably change plant height across environmental heterogeneity. However, our study suggests that there are different QTLs for different developmental time points, and thus, timing of trait measurement needs to be carefully considered in future QTL and breeding studies. Even though there was significant among-genotype variation for number of tillers at all of the time points, we did not map any significant QTLs. Neither did we map any significant QTLs for growth traits that were derived from tiller data, nor did we map any significant QTLs for growth traits that were derived from plant height data. One possible hypothesis for why we did not map QTLs for growth traits is that these traits have a highly polygenic genetic basis with relatively small individual QTL effect sizes.

An upright growth architecture is highly desirable in bioenergy grasses because lodging can make the harvesting of stands difficult [32]. We mapped colocalizing QTLs for tiller angle on March 23 and April 30 of 2012 to LG 8a.

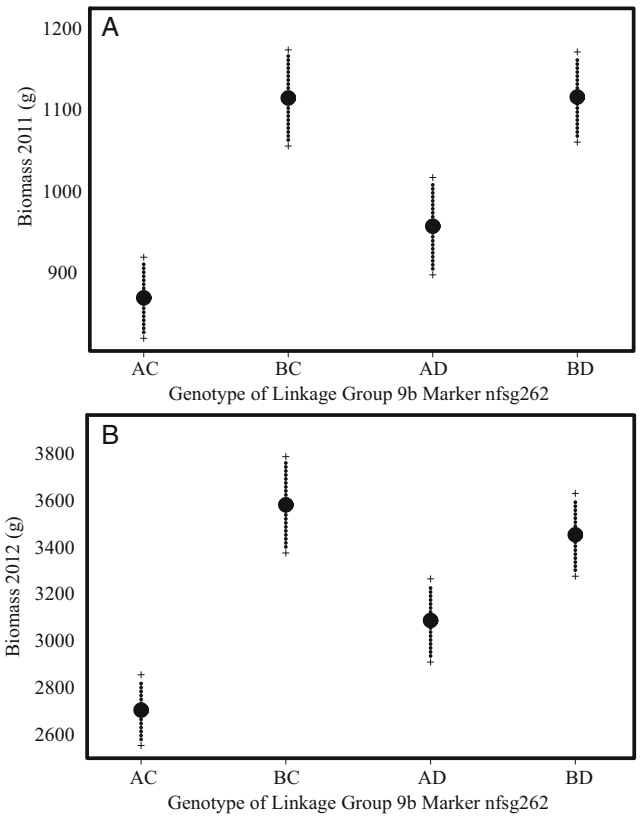


**Fig. 4** Plot for phenotypic effects of genotypes at the nfsg262 marker of linkage group 9b on plant biomass in **a** 2011 and **b** 2012. A and B alleles are derived from Alamo-A4, while C and D alleles are derived from Kanlow-K5. Means and standard errors are plotted for each genotype

◀ **Fig. 3** Combined outbred linkage map with location of quantitative trait loci (QTLs). LOD (1.5) drop confidence intervals of QTLs for traits measured in 2011 (*white*) and 2012 (*gray*) are plotted. *TMass* tiller mass, *InWidth* internode width, *HeadDate* heading date, *TLn* tiller length, *TWidth* tiller width, *NWidth* node width, *PlHt* plant height, *InLn* internode length, *CrWidth* crown width, *LfColor* leaf color, *PHR* post-harvest regrowth, *TAngle* tiller angle, *FLfWidth* flag leaf width

**Table 3** Significant quantitative trait loci discovered in the Albany population

| Year | Trait | Linkage group | Position | CI | LOD | %var | BC | AD | BD |
|---|---|---|---|---|---|---|---|---|---|
| 2011 | Biomass (g) | 9b | 83 | 48–96 | 4.431 | 10.285 | 247.131 | 80.940 | 250.032 |
| 2011 | Chlorophyll content (SPAD) | 9b | 195.85 | 174–195.85 | 4.524 | 7.932 | −1.569 | −0.106 | −1.874 |
| 2011 | Crown width (cm) | 5a | 108 | 98–124.45 | 4.379 | 9.556 | 0.644 | −1.882 | −0.619 |
| 2011 | Leaf color (1–5) | 5b | 97 | 30–103 | 4.428 | 9.916 | −0.211 | −0.301 | −0.392 |
| 2011 | Leaf color (1–5) | 6b | 9 | 0–19 | 4.526 | 10.463 | −0.147 | −0.026 | −0.378 |
| 2011 | Plant height April 13, 2011 (cm) | 3b | 58 | 0–74 | 4.436 | 10.068 | −5.826 | 4.185 | −0.078 |
| 2011 | Plant height April 3, 2011 (cm) | 3b | 61 | 0–68.22 | 5.366 | 12.063 | −3.905 | 7.052 | −0.385 |
| 2011 | Plant height May 26, 2011 (cm) | 2a | 63.17 | 51–125.37 | 4.605 | 10.472 | 7.827 | −5.174 | 0.340 |
| 2011 | Plant height August 12, 2011 (cm) | 4a | 37 | 15–65 | 4.667 | 10.521 | 12.412 | 3.518 | 14.472 |
| 2011 | Second internode length (cm) | 2b | 8 | 0–22 | 4.215 | 8.216 | −0.873 | −0.220 | −0.804 |
| 2011 | Second internode length (cm) | 9b | 89.7 | 48–155 | 4.441 | 7.858 | 0.313 | 0.453 | 1.035 |
| 2011 | Second internode width (mm) | 1b | 34.02 | 22–76 | 4.720 | 10.703 | 0.078 | 0.392 | 0.349 |
| 2012 | Base tiller width (mm) | 1b | 38 | 12–67 | 5.972 | 13.172 | −0.066 | 0.366 | 0.314 |
| 2012 | Biomass (g) | 9b | 84.06 | 56–100 | 5.401 | 12.029 | 808.280 | 494.976 | 817.418 |
| 2012 | First node width (mm) | 1b | 38 | 3–76 | 6.037 | 13.299 | 0.064 | 0.534 | 0.496 |
| 2012 | Flag leaf width (mm) | 8b | 90 | 80–99.97 | 4.578 | 8.916 | 1.718 | −1.448 | −3.523 |
| 2012 | Fourth leaf area ($cm^2$) | 1b | 50.91 | 34.02–73 | 4.575 | 9.889 | 3.323 | 10.491 | 7.153 |
| 2012 | Fourth leaf length (mm) | 1b | 56.84 | 35–73 | 5.994 | 12.346 | −0.824 | 6.406 | 3.306 |
| 2012 | Fourth leaf length (mm) | 2a | 72 | 60–86 | 5.319 | 11.598 | 2.672 | −4.114 | −1.892 |
| 2012 | Heading date (Julian day) | 1b | 100 | 47–109 | 4.589 | 9.114 | −9.691 | −0.227 | 1.207 |
| 2012 | Mean tiller mass (g) | 1a | 31 | 23–41 | 5.695 | 12.718 | 2.176 | 0.693 | 4.497 |
| 2012 | Mean tiller mass (g) | 1b | 41.27 | 34.02–82 | 5.493 | 12.052 | 0.554 | 4.369 | 2.976 |
| 2012 | Plant height April 18, 2012 (cm) | 9b | 76 | 61–93 | 4.530 | 9.574 | 9.959 | 4.906 | 10.240 |
| 2012 | Post-harvest regrowth | 5b | 109 | 101–116 | 6.015 | 12.822 | 1.696 | 0.233 | 1.430 |
| 2012 | Tiller angle March 23, 2012 | 8a | 51 | 30–75 | 4.618 | 10.501 | 0.820 | 0.010 | 0.629 |
| 2012 | Tiller angle April 30, 2012 | 8a | 22 | 7–63 | 4.633 | 9.999 | 0.860 | 0.521 | 0.575 |
| 2012 | Tiller length (cm) | 1b | 55.72 | 34.02–86 | 4.716 | 8.784 | −1.318 | 11.802 | 8.031 |

*CI* confidence interval, *LOD* log of odds, *%var* percent of phenotypic variance explained by QTL, *BC, AD, BD* the additive effects of the BC, AD, or BD genotypes relative to the mean of the AC genotype

Segregating variation in Alamo-A4 primarily caused this QTL. The consistent finding of a tiller angle QTL on LG 8a suggests that it might be useful for breeding of an upright growth architecture.

We identified two QTLs (LG 5b and LG 6b) that contributed to leaf color. Variation in leaf color among switchgrass plants at least partially reflects variation in leaf wax composition. Leaf waxes can have the function of preventing water loss and can be important for drought tolerance [33]. We also identified a QTL for chlorophyll content (measured by SPAD) on LG 9b, which did not colocalize with either of the leaf color QTLs.

We identified a QTL for post-harvest regrowth located on LG 5b. Variation in post-harvest regrowth was striking, with some plants producing new leaves on a majority of tillers, while others were completely dormant. This variation may reflect differences in phenology of the plants, with some plants becoming dormant earlier in the fall. Variation in regrowth could also reflect differences in the tolerance of plants to harvesting, which would be a beneficial trait for biomass production over consecutive seasons or in multiple cut harvesting schemes.

We detected no significant genotype × environment QTLs by mapping on the difference in trait values between the wet and dry treatment in 2012. The lack of these QTLs could be due to the weak overall treatment effects, for which we observed significant effects for only four traits (described above).

## A Common Locus of Biomass Variation?

Recent studies have demonstrated that large gains in biomass yield are possible after only a few generations of mass selection [34, 35]. However, breeding efforts have yet to take advantage of marker-assisted selection [35, 36]. One of the most significant results of this study was mapping of the biomass QTL on linkage group 9b that was consistently found across years. The largest (LOD=6.8) biomass QTL discovered by Serba et al. [16] was in a similar region of linkage group 9b and had a similar sized additive effect,

accounting for 18.1 % of the average biomass. In contrast to our study, the large biomass QTL of the Serba et al. study was caused by variation harbored within Summer-VS16. The results of Serba et al. [16] and our study suggest that segregating variation for biomass could be frequently located on this portion of linkage group 9b. Future studies could very inexpensively genotype this region on linkage group 9b with a handful of existing markers (sww2524, nfsg262, sww25, nfsg283, etc.) in any hybrid mapping population. If an association were detected, then those same markers could be used for marker-assisted breeding.

## Conclusions and Future Directions

Our study identified QTLs for biomass and multiple other potentially useful traits for improvement of switchgrass. Based on this study, markers from the regions of discovered QTLs can now inexpensively be used to conduct targeted genotyping in other mapping populations to establish direct links between genomic regions and important phenotypes for breeding. This is especially true for markers in the vicinity of the biomass QTLs on LG 9b in both our study and that of Serba et al. [16]. Further, comparative mapping with other closely related species, including *Setaria italica* and *Panicum hallii*, has great potential to uncover common loci that are responsible for within-species variation in important traits [37, 38].

Our study and the study of Serba et al. [16] were both limited by the full-sib mapping population design, which is only capable of identifying QTLs caused by within-individual variation. We have recently constructed a four-way cross-mapping population with two southern lowland and two northern upland parents. The genetic divergence between upland and lowland ecotypes and the clines found along the latitudinal gradient across North America represents large sources of phenotypic variation in switchgrass [21]. We expect that our four-way cross-mapping population will allow us to identify QTLs involved in key regional adaptations, between the upland and lowland ecotypes, and across latitudes.

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