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ORIGINAL RESEARCH

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High-density linkage map reveals QTL underlying growth traits in AP13×VS16 biparental population of switchgrass

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Abstract

Switchgrass (*Panicum virgatum* L.), a native warm-season perennial grass, is being considered as a feedstock for biofuel production in the United States. To expedite its genetic improvement and enhance genetic gain per selection cycle, application of marker-assisted selection is indispensable. A high-density linkage map was constructed in a pseudo-F_1 testcross mapping population of AP13×VS16, consisting of 349 progenies. A total of 8,757 single nucleotide polymorphism (SNP) markers generated through genotype-by-sequencing (GBS) were used to construct the linkage map. The total map length spans up to 2,540.2 cM with the marker density of one marker in every 0.25–0.34 cM. Spring green-up (SG), days to flowering (FL), and the vegetative growth period (VP) data were analyzed and used for quantitative trait loci (QTL) mapping. The population showed significant variations and exhibited transgressive segregation for SG, FL, and VP. QTL analyses were performed using trait mean of each year and location along with BLUP (best linear unbiased prediction) values of the traits. A total of 35, 37, and 34 QTL for SG, FL, and VP, respectively, were identified. Phenotypic variability explained by each QTL ranged from 11.29% to 27.85%. The additive genetic effects of individual QTL ranged from −1.81 to 2.40, −6.12 to 7.58, and −16.01 to 6.38 for SG, FL, and VP, respectively. Comparing major QTL regions in the switchgrass genome, 20 candidate genes were identified which were reported to be involved in growth-, development-, and flowering-related traits in switchgrass.

KEYWORDS

flowering, linkage map, QTL, spring green-up, Switchgrass, vegetative growth period

1 | INTRODUCTION

Switchgrass (*Panicum virgatum* L.) is a vigorous warm-season (C4) perennial grass predominantly grown in the tall grass prairie of North America. It is widely distributed in east of the Rocky Mountains in the United States and generally south of the 55° north latitude (Stubbendieck, Hatch, & Butterfield, 1992). Compared with many other perennial grasses and conventional crops, switchgrass has high biomass yield potential and high cellulose content (Rinehart,

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2006). Relatively high and reliable productivity across a wide geographical range, low input requirement, and suitability in marginal land that is too dry and uncultivable for other crops (Wright & Turhollow, 2010), makes switchgrass a preferred second-generation (lignocellulosic) feedstock for bioenergy production.

Highly interrelated traits, such as spring green-up (SG), flowering (FL), and vegetative growth period (VP), contribute directly to biomass production. In annual crop species, early planting enhances biomass production by extending VP (Danalatos & Archontoulis, 2010). However, in a perennial species like switchgrass, early spring green-up after long winter dormancy plays a significant role in biomass production.

Days to flower is an important agronomic trait, which plays a key role in the adaptation and geographic distribution of switchgrass ecotypes. The upland ecotypes are late in spring green-up and early in flowering which enables them to adapt to the short growing season in the higher latitude areas of the United States. On the other hand, the lowland ecotypes green-up early in the spring and are late in flowering. Flowering time plays a key role in biomass yield with one day delay increased biomass yield by 0.47 Mg/ha in lowland switchgrass ecotype (Casler, 2014). Negative correlations have been reported between biomass yield and maturity (Newell, 1968; Talbert, Timothy, Burns, Rawlings, & Moll, 1983), indicating that late flowering cultivars accumulate more biomass. Therefore, identification of the QTL underlying flowering in population of a lowland × upland cross would have a significant impact in switchgrass breeding as a tool for trait enhancement.

Vegetative growth period was reported to have an essential impact on biomass yield (Demura, Ye, & Ye, 2010). Biomass yield of switchgrass increased with the increase of VP duration (Van Esbroeck, Hussey, & Sanderson, 1998). The number of days between the SG and FL actually represents the VP, where nearly all of the biomass accumulation takes place. Longer generative and vegetative growth period, faster growth rate, and greater response to input contributed to greater biomass yield (Kusmenoglu & Muehlbauer, 1998).

Early SG and late FL in switchgrass are the likely characteristics to extend the VP that contribute to the biomass accumulation. The VG also substantially increases grazing period while maintaining the forage quality. The shift from the vegetative to the reproductive growth signifies the downhill of the biomass accumulation because the photosynthetic products are mostly used to complete the development of reproductive parts. Dry matter digestibility and concentrations of crude protein, ADF (acid detergent fiber), NDF (neutral detergent fiber), and cellulose decline with the maturity of the switchgrass hay (Burns, Pond, Fisher, & Luginbuhl, 1997; Griffin & Jung, 1983; Twidwell, Johnson, Cherney, & Volenec, 1988).

Marker-assisted selection (MAS) showed great importance to enhance genetic gain in crop improvement. Uniformly distributed and relatively high-density markers in the genome are necessary for effective QTL mapping and marker–trait association studies. Generation of large number of SNPs from genotyping-by-sequencing (GBS) and from whole genome and exome sequencing has proven to be powerful resources employed in the refinement of breeding practices, even for complex polyploid plant species. Genetic linkage map construction, QTL analysis, and genome-wide association studies (GWAS) are being implemented in MAS and genetic selection techniques in molecular plant breeding programs. GBS has been proven to be a very cost-effective and useful method in generating SNP markers that covers whole genome (Elshire et al., 2011; He et al., 2014; Poland, Brown, Sorrells, & Jannink, 2012; Poland & Rife, 2012; Sonah et al., 2013; Spindel et al., 2013). However, the challenges with GBS are the lack of uniform and enough coverage of the genome to call SNPs accurately. In addition, proportion of genotypes with missing markers and their trade-off, and the assignment of markers in proper order within the chromosomes are considered the major hurdles of GBS. Following the GBS method, 1.2 M SNP markers were generated in switchgrass (Lu et al., 2013), but only 3,000 of them could be used in constructing linkage map.

QTL mapping for flowering was reported in major bioenergy crops such as switchgrass (Dong et al., 2015), miscanthus (*Miscanthus* '*Giganteus*' syn. *Miscanthus floridulus*; Atienza, Satovic, Petersen, Dolstra, & Martín, 2003; Jensen, Thomas-Jones, Farrar, Clifton-Brown, & Donnison, 2008), sorghum (*Sorghum bicolor*; Feltus et al., 2006; Mace & Jordan, 2010; Takai, Yonemaru, Kaidai, & Kasuga, 2012; Zou et al., 2012), and maize (*Zea mays*; Zhang et al., 2011). QTL for reproductive maturity traits were identified in chromosomes 1N (1a), 2N(2b), 3K(3a), 3N(3b), 7K(7a), 8K(8b), and 9N(9a) in the NL94×SL93 switchgrass biparental population, as well as in the selfed progenies of NL94 (Dong et al., 2015). QTL with positive additive effects for postharvest regrowth were reported on chromosome 5N (5b) of a switchgrass biparental population, Kanlow-K5×Alamo-A4 (Lowry et al., 2015). Linkage map from the same pseudo-F_1 testcross population was constructed using SSR, STS, and DArT markers, and QTL for biomass yield, plant height, and recalcitrant traits were identified (Serba et al., 2015, 2016, 2013).

Construction of high-density linkage map and identification of genes linked to QTL would have a valuable role in increasing genetic selection gain by employing marker-assisted breeding. The objective of this study was to construct linkage map using SNP markers and to detect the QTL underlying growth traits that contributes to biomass yield, determine their effects and breeding implications. In this study, a high-density linkage map was constructed in a pseudo-F_1 testcross population, AP13×VS16, using

8,757 haplotype SNP markers generated through GBS. It is expected this linkage map will be a valuable resource in resolving some of the bottlenecks of low-density linkage map, especially for finding genes associated with QTL accurately. However, major challenges are how to integrate large number of markers in constructing linkage map and subsequently in analyzing QTL. This study reports important modifications in the procedures for generating linkage map and QTL analysis for switchgrass growth traits, such as SG, FL, and VP.

2 | MATERIALS AND METHODS

2.1 | The mapping population

A pseudo-F_1 testcross mapping population was developed by crossing two heterozygous genotypes, AP13 and VS16. AP13, the female parent, was a selection from a lowland cultivar 'Alamo' (Casler, 2014; Serba et al., 2013). AP13 is characterized by early regrowth in the spring, late heading, tall plants up to 270 cm, strong shoot that withstands lodging, and high biomass yield. VS16, the male parent, was a selection from the upland cultivar 'summer.' It is characterized by late regrowth, early heading, short plant height with slender stem, and low biomass yield. The population was originally developed at the University of Georgia (Missaoui, Paterson, & Bouton, 2005) and further expanded at the Noble Research Institute (Serba et al., 2013). A total of 349 pseudo-F_1 progenies of the population were used for linkage map construction of which 251 were used for phenotyping. Genotypes with significant missing markers were excluded, and a final set of 120 genotypes were used for linkage map construction and QTL analysis. The selected genotypes had at least 50% of the 14,212 markers.

2.2 | Field experiments and phenotypic data

Field experiments were conducted at two Oklahoma locations: Ardmore (Ard) and Red River research farm near Burneyville (RR), which were planted on July 19, 2007, and May 08, 2008, respectively. The experiments were laid out in an R-256 honeycomb design (Fasoulas & Fasoula, 2010) with four replications. Details of field establishment and management practices were described previously (Serba et al., 2015, 2013).

Data on SG and FL were collected from Ardmore during 2008–2011 and from Red River during 2009–2011. Spring green-up dates were recorded for each plant at the time of new shoot emergence from the crown during spring. Days to flower was recorded when 50% tiller of a plant had inflorescence. Spring green-up and FL dates were converted to calendar days with the help of 'Days360' function in Excel using the first day of the year as a starting date. Vegetative growth period was calculated as days from SG to FL.

Analysis of variance was conducted using PROC GLM procedure in SAS 9.3® (SAS Institute, Cary, NC, USA). Genotype, genotype × location, genotype × year, and genotype × location × year were considered as random effects. Statistical significance was determined at $p \leq 0.05$. Frequency distribution of each of the traits was plotted for year-location combinations to check normality of the data. Correlation coefficients among SG, FL, and VP were calculated across all location-year using PROC CORR procedure in SAS 9.3® (SAS Institute).

2.3 | Marker development, genetic linkage map construction, and QTL analysis

Young leaf tissues from parents and F_1 pseudotestcross progenies were collected in a 2-ml tube and froze in liquid nitrogen. Samples were ground to fine powder. DNA was extracted using a DNeasy® Plant Mini-prep DNA Extraction Kit (QIAGEN Inc., Valencia, CA, USA) following manufacturer's instructions. DNA concentrations were measured using a NanoDrop ND 1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). GBS library preparation and sequencing were performed at Joint Genome Institute facility, Walnut Creek, CA, with 2 × 100 bp pair end run set in HiSeq2000. The sequence coverage was ½x to 8x. A set of 349 genotypes (out of 367) had enough sequence data to call meaningful SNPs.

SNP calling used an initial set of AP13-specific (Switchgrass v4.0 reference genome) intrachromosomal specific 51-mer markers to segregate the 18 switchgrass chromosomes. The criteria for the 51-mers was that: (a) They were found only in AP13 and not in VS16 and (b) the 52nd base that immediately follows the 51-mer segregates appropriately in the F_1 progenies. The 52-mers were then counted in the progenies. The linkage map was then constructed representing the two subgenomes combining the data from both AP13 and VS16 parents. A set of 455,999 haplotype markers were selected based on their segregation ratios and number of uncalled progenies. The final marker calls were phased into separate subgenomes, and the phasing was tested against a set of ~5,000 BAC clones. The error rate in phasing was estimated to be 0.41%.

A marker set of 14,212 were used in constructing genetic linkage map for each of the 18 chromosomes initially. The maximum likelihood method of JoinMap 4.1 (www.kyazma.nl) was used to order the markers within the chromosomes and to calculate initial map distances using haplotype (HAP) model. Map distances were then adjusted to Kosambi map function according to the formula suggested in JoinMap 4.1. As WinQTLCartographer (Wang, Basten, & Zeng, 2012) has limitation of 500 markers for each chromosome, thus linkage map was constructed with 8,757 markers—500 markers from

TABLE 1 Analysis of variance of traits of interest in AP13×VS16 mapping population of switchgrass evaluated at Ardmore and Burneyville, OK, during 2008 through 2011 growing seasons

Source	*df*	Mean square		
		SG	FL	VP
Genotype	119	48.41*	193.51*	220.61*
Location	1	5,603.71*	39,386.42*	55,399.78*
Year	3	91,652.65*	64,437.77*	10,527.09*
Genotype × location	119	17.32*	165.82*	168.24*
Genotype × year	357	9.71*	91.34*	87.82*
Location × year	3	6,767.13*	13,168.25*	21,257.79*
Genotype × location × year	357	8.97NS	84.66*	92.57*
Residual	2,306	7.79	32.81	39.20

FL: days to flowering; NS: nonsignificant; SG: spring green-up; and VP: vegetative growth period.
*Statistically significant at 0.01 probability.

each chromosome with the exception of chromosome 7N that contains only 257 markers. In selecting markers, the distance of markers from neighboring ones was first calculated using map distance of original map. Then, markers that are very close were removed until a maximum of 500 markers were obtained for each chromosome. This procedure ensures marker sets that were almost evenly distributed within the chromosome.

QTL mapping was conducted using Windows QTL Cartographer v2.5 (Wang et al., 2012) considering the means as well as the BLUP values of the traits. Identification and reporting of the QTL were done using the composite interval mapping (CIM) approach (Zeng, 1994). Genome-wide logarithm of odds (LOD) thresholds of 2.5 was used to call for QTL. The cofactor markers were determined as implemented in standard CIM model using the forward–backward regression method. A walking speed of 2 cM in windows size of 10 cM was used. The physical map of switchgrass genomes flanking 50 kb up- and downstreams of the major QTL peak markers was scanned, and annotated genes within these regions were identified using switchgrass v4.1 annotation information.

3 | RESULTS

3.1 | Phenotypic variations

Analysis of variance (ANOVA) showed significant variations among the genotypes of F_1 pseudotestcross mapping population for SG, FL, and VP (Table 1). In addition to the genotypic variation, there was significant genotype × environment interaction of these traits. Frequency distribution of the data revealed nearly normal distribution for all the traits in all location-year combinations (Figures 1–3). At the same time, transgressive segregation was prominent in all location-year combinations. Transgressive segregation could not be called for FL or VP in RR2011 since parental data were not available for these two environments. The mean SG for the population ranged from 58.8 to 89.1 days (Table 2). The earliest and the

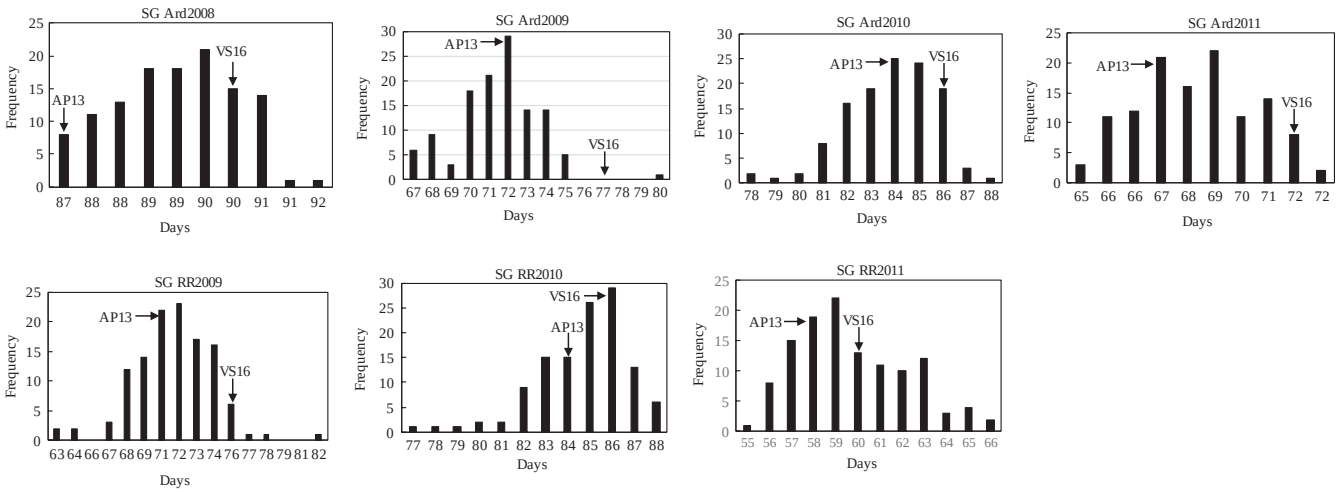


FIGURE 1 Frequency distributions of spring green-up (SG) in the population at Ardmore (Ard) and Burneyville (RR) during 2008–2011 growing seasons

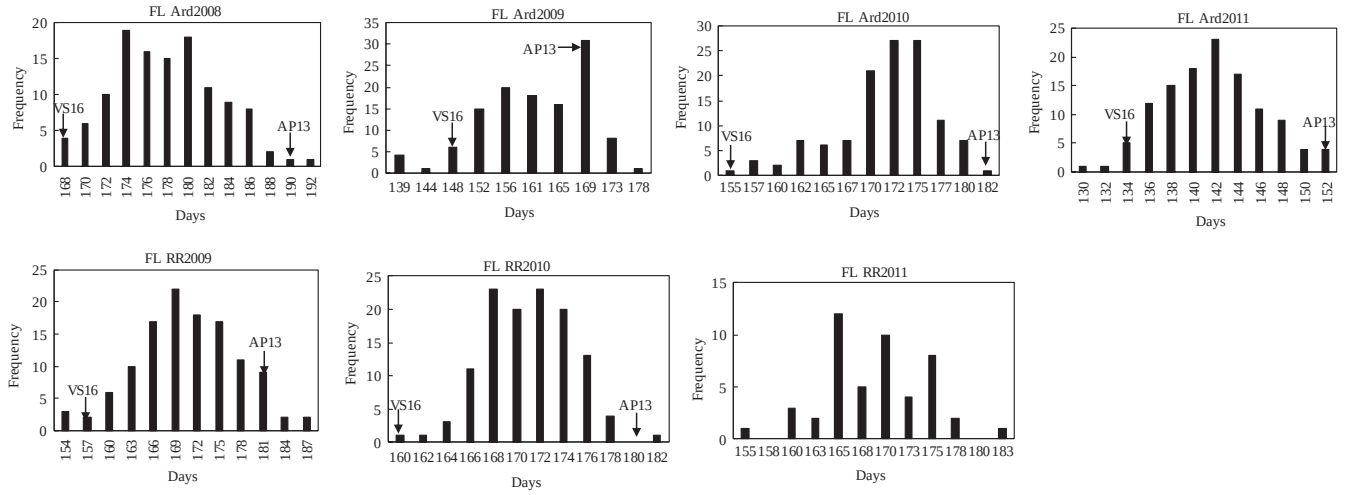


FIGURE 2 Frequency distributions of days to flower (FL) in the population at Ardmore (Ard) and Burneyville (RR) during 2009–2011 growing seasons

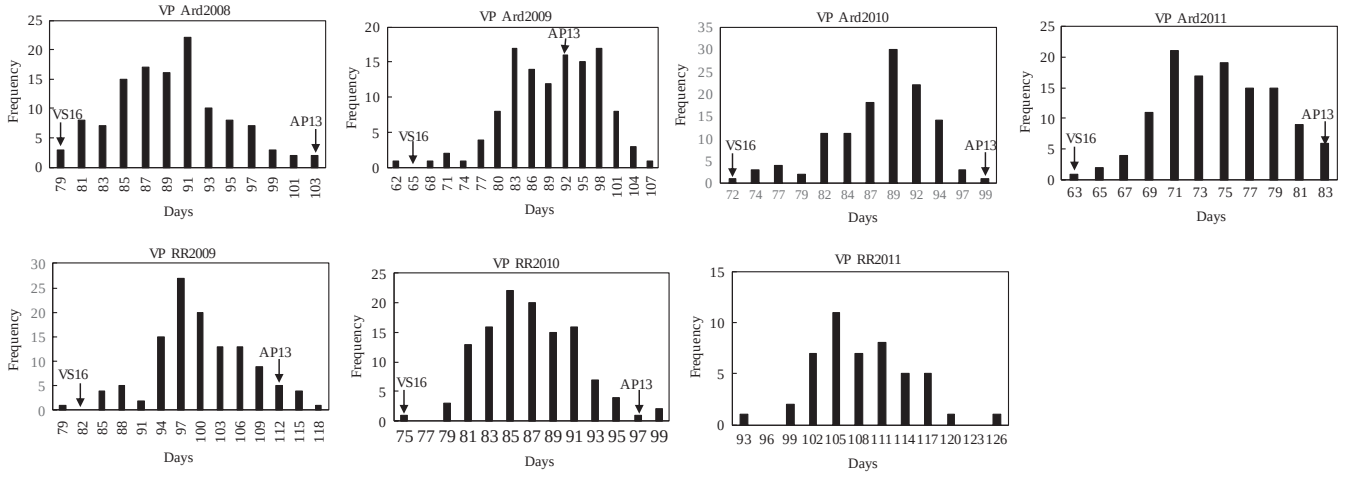


FIGURE 3 Frequency distributions of vegetative growth period (VP) in the population at Ardmore (Ard) and Burneyville (RR) during 2009–2011 growing seasons

latest SG were observed at RR in 2011 and Ardmore 2008, respectively. The mean FL ranged from 140.8 to 177.1 days, whereas VP ranged from 72.9 to 107.4 days. Significant correlations were observed between FL and VP across all location-year with coefficients values ≥0.83 (Supporting information Table S1). SG and VP were negatively correlated.

3.2 | Genetic linkage map construction

The linkage map used for QTL detection constructed of 8,757 markers arranged in 18 chromosomes with a maximum of 500 markers in each chromosome. Map length varies from 67.5 to 168.4 cM (Figure 4, Table 3). One marker was present in 0.25 to 0.34 cM, which means 2.97 to 4.06 markers present in every cM of the map distance. List of markers with map position for original and new linkage map is provided in Supporting information Tables S2 and S3, respectively. Estimation of pairwise recombination fraction showed almost perfect positioning of the markers on the chromosomes. There was no marker displacement found for any chromosome in the recombination fractions plot (Figure 5).

3.3 | QTL analyses

A total of 106 QTL were recorded for all the three growth traits with a LOD of ≥2.5. A higher number of QTL (72) were associated with Ardmore location as compared with RR (34) (Supporting information Table S4). Chromosome 3K harbored the highest number of QLT (15) followed by chromosomes 2K (12) and 1K (11) (Supporting information Table S5). One QTL was identified in each of the chromosomes 3N, 4K, 4N, and 8K.

TABLE 2 Mean phenotypic trait values for AP13, VS16, and 120 pseudotestcross progeny evaluated at two Oklahoma locations during 2008–2011

Environment	Parents		Population	
	AP13	VS16	Mean ± *SD*	Range
Spring green-up				
Ard2008	65	90	89.1 ± 1.7	84–94
Ard2009	72	77	71.2 ± 3.0	60–84
Ard2010	84	86	83.4 ± 3.0	55–91
Ard2011	67	72	68.0 ± 2.7	57–74
RR2009	70	76	70.7 ± 4.0	59–95
RR2010	84	86	84.3 ± 3.2	66–92
RR2011	59	61	58.8 ± 3.9	52–83
Days to flower				
Ard08	190	166	177.1 ± 6.2	165–193
Ard09	164	141	159.1 ± 10.0	121–180
Ard10	183	151	170.0 ± 7.5	126–184
Ard11	160	134	140.8 ± 5.3	127–155
RR2009	181	156	168.7 ± 10.4	133–196
RR2010	180	154	170.2 ± 4.7	156–184
RR2011	–	–	168.4 ± 6.4	154–184
Vegetative growth period				
Ard2008	125	76	88.0 ± 6.4	74–106
Ard2009	92	64	87.9 ± 10.0	47–114
Ard2010	99	65	86.6 ± 7.7	39–114
Ard2011	93	62	72.9 ± 5.5	57–87
RR2009	111	80	98.1 ± 11.2	67–128
RR2010	96	68	85.9 ± 5.7	72–105
RR2011	–	–	107.4 ± 7.3	90–126

Note. Ard: Ardmore, RR: Red River Research Farm near Burneyville, OK.

Thirty-five QTL were detected for SG in seven location-year combinations (Table 4). Eight QTL showed negative additive effects, while 27 had positive effects, an indication of abundance of late-spring green-up QTL in the population. The proportion of phenotypic variability explained (PVE) by the QTL ranged from 11.29% to 27.85%. Among the negative effect QTL, SG_9N_57447010 showed the highest PVE of 19.37 with additive effect of −0.73. The positive additive effect of the QTL ranged from 0.63 to 2.40, while the negative effect from −0.73 to −1.81. Five QTL showed at least 15% PVE and all had negative additive effects.

A total of 37 QTL for FL were detected (Table 5). The PVE of the FL-related QTL ranged from 11.31% to 26.95%. Twenty-four QTL had PVE value more than 15%: Eight of them had positive additive effects, indicating their influence on late flowering. The QTL, FL_3K_11371679, showed the highest PVE of 26.95% with positive allele effects of 3.69. In general, the PVE values of the QTL detected at Ardmore, especially in 2008 and 2011, were higher than those for the other year-locations.

Thirty-four QTL were recorded for VP (Table 6) with PVE values ranging from 11.49% to 23.10%. Eighteen QTL showed PVE more than 15% and seven had positive additive effects, which indicates long vegetative growth period that can enhance biomass production. The highest positive additive effect was observed for the QTL VP_2K_67003274, whereas VP_8N_40657439 showed the highest negative additive effect. VP_9N_63132454 and VP_9N_53557827 were colocalized in Ardmore for the year 2009 and 2011.

3.4 | QTL for BLUP values

Based on the significant genotype × environmental interactions, BLUP values for all three traits were estimated for each

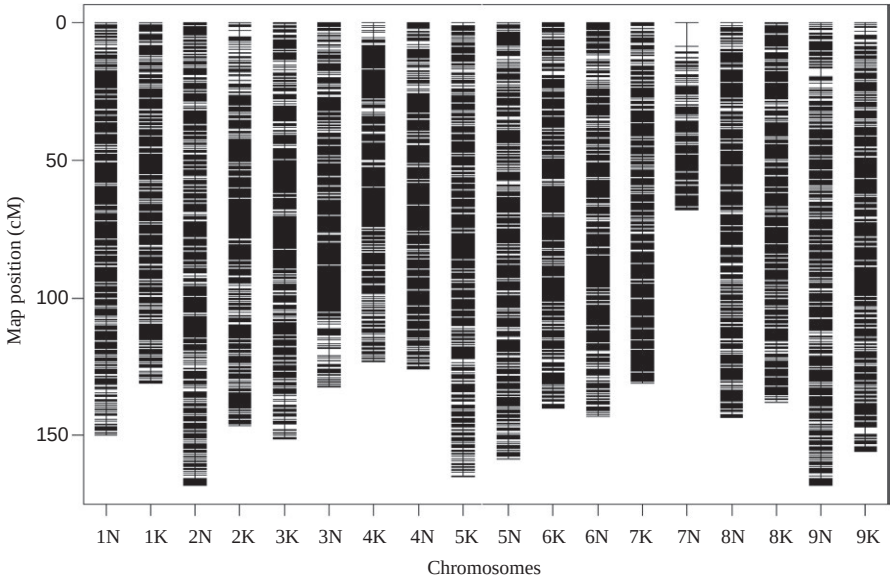


FIGURE 4 Distribution of 8,757 haplotype SNP markers in 18 switchgrass chromosomes. *Y*-axis describes the CentiMorgan distance, and *X*-axis is the chromosome number

TABLE 3 Chromosome size, map length, marker distribution, and densities in each of the 18 switchgrass chromosomes

Chromosomes	1N	1K	2N	2K	3K	3N	4K	4N	5K	5N	6K	6N	7K	7N	8K	8N	9K	9N
Size (Mb)	97.8	80.4	103.9	93.6	73.0	57.8	63.6	56.3	116.5	100.4	72.9	80.1	75.7	70.8	77.8	73.9	88.0	122.8
Markers	500	500	500	500	500	500	500	500	500	500	500	500	500	257	500	500	500	500
Map length (cM)	149.9	131.0	168.2	146.6	151.5	132.4	123.3	126.0	165.2	158.5	139.9	143.0	131.1	67.7	138.0	143.6	155.9	168.4
SNP density	0.30	0.26	0.34	0.29	0.30	0.26	0.25	0.25	0.33	0.32	0.28	0.29	0.26	0.26	0.28	0.29	0.31	0.34
SNPs/cM	3.34	3.82	2.97	3.41	3.30	3.78	4.06	3.97	3.03	3.15	3.57	3.50	3.81	3.80	3.62	3.48	3.21	2.97

of the genotypes from all replication, location, and year data. A total of 16 QTL were identified using the BLUP values of which six were for SG, seven for FL, and three for VP (Table 7). One BLUP QTL of each of the traits appeared at the same position of their corresponding year-location QTL. These are as follows: SG_BLUP_6N_22131630 and SG_6N_20163874 for RR2009 (Tables 4 and 7), FL_BLUP_1K64692294 and FL_1K64692294 for Ardmore 2011 (Tables 5 and 7), and VP_BLUP_1K_57091180 and VP_1K_57091180 for Ardmore 2011 (Tables 6 and 7). LOD score, PVE, and additivity of the BLUP QTL ranged from 2.50 to 9.45, 11.36 to 43.65, and −6.17 to 4.09, respectively (Table 7).

3.5 | Identification of colocalized and pleotropic QTL

QTL that appeared at the same or overlapping positions across year-locations were examined for each of the traits. For SG trait, QTL SG_2N_86467959 and SG_2N_89345610 appeared in both Ardmore 2008 and 2009 at the same position of the genome. QTL SG_5K_6039252 of RR2009 and SG_5K_6040501 of Ard2009 overlap (Table 4). Similarly, QTL for VP, VP_3K_3071522, were detected both in Ard2009 and in RR2011 (Table 6). Detection of the same QTL in different environmental conditions indicates the reliability of this QTL in MAS.

After scanning common QTL among all three traits, four QTL were identified that are associated with both FL and VP traits. These colocalized or pleiotropic QTL are FL/VP_1N_6206723, FL/VP_1K_60899297, FL/VP_2K_7303895, and FL/VP_7K_12994974 and they are located on chromosomes 1N, 1K, 2K, and 7N (Tables 5 and 6), respectively. Two colocalized BLUP QTL were identified for FL and VP, FL/VP_BLUP_2N_23594225, and FL/VP_BLUP_3K_14668834.

3.6 | Mapping and annotation of the major QTL

We mapped 13, 13, and 9 QTL which had at least 15% PVE and typical QTL peaks for SG, FL, and VP, respectively, on switchgrass chromosomes (Figure 6). These were considered as major QTL. QTL box and whisker values were calculated as width of QTL at 1 and 2 LOD values down from peak marker LOD scores, respectively. Chromosome 2N possessed the highest number of major QTL (5 for SG and 1 for FL, Figure 6). Chromosomes 1K and 2K harbor 4 QTL each for FL and VP. Four QTL were also found on 3K (2 SG, 1 Fl, and 1 VP (Figure 6).

We reported annotation of 363 genes identified within 50 kb upstream or downstream regions from the peak marker of 10, 12, and 8 QTL for SG, FL, and VP traits, respectively (Supporting information Table S6). These QTL had at least one annotated gene. QTL RG_3K_7236072

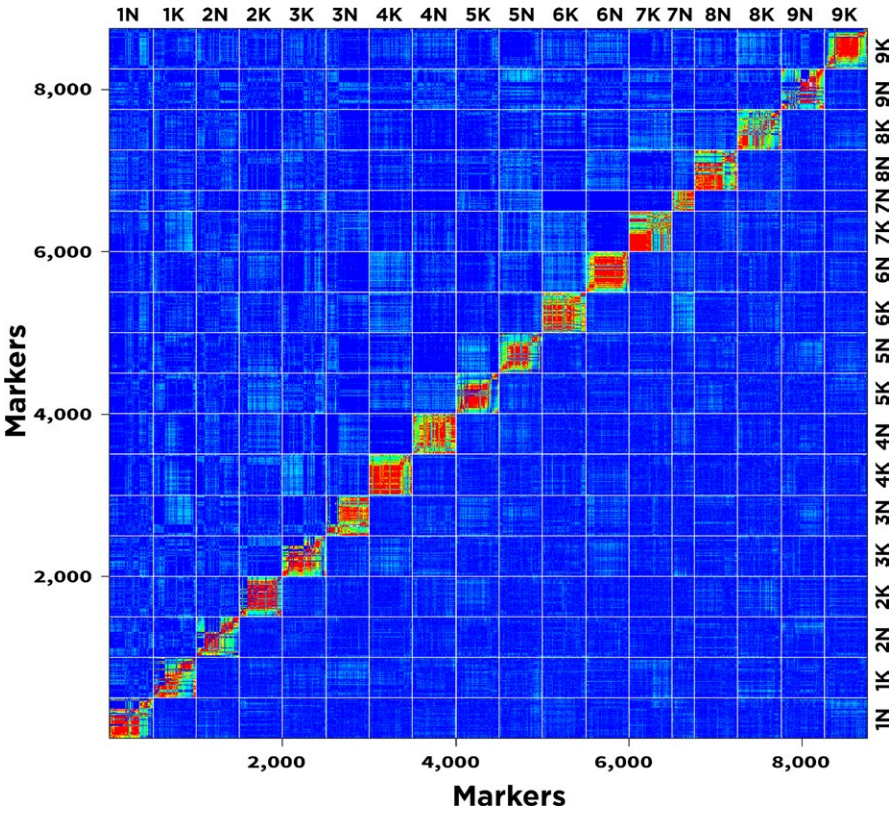


FIGURE 5 Plots of estimated pairwise recombination fractions (upper triangles on the diagonal) and LOD scores (lower triangles on the diagonal) for all the markers. Red indicates very low recombination with high LOD values, and blue indicates very high recombination (unlinked) with low LOD value

(Ard2010) had the highest 23 annotated genes followed by QTL FL_3K_11371679 (Ard2008) with 21 annotated genes and QTL FL_3K_8820134 (Ard2008) possesses 19 genes. QTL RG_6K_18323886 (Ard2010) had only one annotation. Twenty genes were identified that are involved directly or indirectly in growth and development, flowering, and a number of biosynthetic pathways (Table 8). Senescence regulator (*REG*), *CELLULASE,* and *WRKY* transcription factors were found associated with QTL for SG in chromosome 2N. The 5K harbors Photosystem II lipoprotein (*Psb*) and *MADS* box transcription factor that were also associated with SG QTL. No apical meristem (*NAM*) protein is localized with SG QTL in chromosome 9N. Flavonoid 3′,5′-hydroxylase (*F3′5′H*) and ethylene-insensitive protein 3 (*EIN3*) were associated with FL QTL, located in 7K and 7N, respectively.

4 | DISCUSSION

4.1 | Parental genotypes and population variability

The two parental genotypes crossed to generate the population for this study greatly varied for all the traits under study. AP13, the female parent, belongs to lowland ecotype and was early in SG, late in FL, and has longer VP. Contrastingly, VS16, the male parent, belongs to upland ecotype and was late in SG, early in FL, and has a short VP. Both the parents are heterozygous due to the obligate outcrossing behavior of the species. The ultimate bioenergy goal of switchgrass breeding is to improve lignocellulosic biomass feedstocks for biofuel production. Three interrelated traits, SG, FL, and VP, play significant contributions to increase biomass production by early green-up in spring and delay in flowering, which increase VP. In switchgrass, even one day delay in flowering increases biomass yield by ~0.47 Mg/ha (Casler, 2014). Early heading shifts the vegetative regeneration into reproductive growth and literally stops biomass accumulation. Thus, the genotypes with early green-up coupled with late heading get longer vegetative growth period to boost their biomass yield.

Frequency distributions of SG, FL, and VP values in the population showed a normal distribution which is characteristic of quantitative traits. The variance component analysis revealed that the effects of environment and genotype × environment interaction are important. The low level of genotypic effect implies that this set of traits is controlled by many genes that have small contribution to the phenotype and largely influenced by the environment and genotype × environment interaction. When parents with many heterozygous loci are crossed, the genetic segregation can be observed in a full-sib family, which is the outcome of genetic recombination of alleles in the two parents during meiosis (Butcher et al., 2002). As a result, number of different segregation types with unknown linkage phases arise in the progenies (Lu, Cui, & Wu, 2004). Genotype means of progenies were lower or higher than the parental mean values. Progeny with earlier SG and later FL than AP13 were observed, demonstrating

TABLE 4 QTL for spring green-up in the AP13×VS16 full-sib population grown at two Oklahoma locations detected using composite interval mapping

SL	QTL	Chr.	Map position (cM)	LOD	PVE (%)	Additivity	Location-year
1	SG_2N_81930337[a]	2N	120.23	3.32	15.44	−1.22	Ard2008
2	SG_2N_86467959[a]	2N	137.49	5.42	25.14	1.59	Ard2008
3	SG_2N_89345610[a]	2N	143.55	4.55	21.03	1.26	Ard2008
4	SG_5K_97754302[a]	5K	128.13	3.71	17.26	0.68	Ard2008
5	SG_5K_106518127	5K	144.90	3.13	14.81	0.63	Ard2008
6	SG_9N_57447010[a]	9N	35.40	4.15	19.37	−0.73	Ard2008
7	SG_2N_86467959	2N	137.49	4.93	23.08	1.59	Ard2009
8	SG_2N_89345610	2N	143.55	3.88	18.16	1.43	Ard2009
9	SG_5K_1714818	5K	0.79	3.60	16.74	1.36	Ard2009
10	SG_5K_6040501[a]	5K	9.06	4.06	19.00	1.45	Ard2009
11	SG_3K_5472751[a]	3K	11.66	4.64	21.37	1.37	Ard2010
12	SG_3K_7236072[a]	3K	20.28	3.27	15.31	1.31	Ard2010
13	SG_5N_93134395	5K	74.84	2.55	11.83	1.00	Ard2010
14	SG_6K_18323886	6K	46.05	3.47	16.10	−1.12	Ard2010
15	SG_2N_33173159	2N	85.19	2.60	12.07	−1.00	Ard2011
16	SG_3N_22787548	3N	93.56	3.35	15.67	−1.13	Ard2011
17	SG_6N_35869963	6N	142.46	3.35	15.61	−1.14	Ard2011
18	SG_8N_11087964	8N	13.06	3.30	15.42	1.22	Ard2011
19	SG_8N_7678596	8N	17.50	3.13	14.50	1.14	Ard2011
20	SG_8N_8605027	8N	37.32	2.67	12.49	1.08	Ard2011
21	SG_8N_46034744	8N	60.35	2.84	12.97	1.10	Ard2011
22	SG_8N_64941793	8N	132.91	2.74	12.78	1.08	Ard2011
23	SG_1K_39581393	1K	59.44	2.71	12.60	−1.64	RR2009
24	SG_5K_439134	5K	1.15	4.52	21.09	2.40	RR2009
25	SG_5K_6039252[a]	5K	9.76	4.52	20.92	2.21	RR2009
26	SG_6N_20163874	6N	47.09	3.08	14.34	1.89	RR2009
27	SG_6N_34086811	6N	84.20	2.50	11.38	1.65	RR2009
28	SG_8K_25645255	8K	83.01	2.50	11.29	1.15	RR2010
29	SG_9K_77802495	9K	16.27	2.93	13.73	1.26	RR2010
30	SG_9K_39115206	9K	97.26	3.15	14.69	1.45	RR2010
31	SG_1K_4650497	1K	17.79	2.63	12.69	1.47	RR2011
32	SG_1K_6848424	1K	20.68	5.98	27.85	1.98	RR2011
33	SG_4N_25850796[a]	4N	74.45	3.44	15.98	1.48	RR2011
34	SG_9N_52464184	9N	74.45	3.20	14.83	−1.81	RR2011
35	SG_9K_6102052[a]	9K	146.47	4.12	19.36	1.59	RR2011

Note. Chr.: chromosome; LOD: logarithm of odds; PVE: phenotypic variance explained.
[a]QTL annotated.

transgressive segregation for these traits. Such transgressive segregations were observed in wheat when favorable alleles were contributed by both parents or by linkage between favorable and unfavorable loci breaks (Mengistu et al., 2012). In either case, the combination of favorable alleles brings about superior performance in a progeny derived from hybridization of genetically diverse parents.

4.2 | Linkage map and genomic regions underlying growth

Quality and density of linkage map are very important precondition for effective QTL mapping. QTL mapping using low-density map has been considered as a factor hindering the identification of precise number and locations of

TABLE 5 QTL for days to flower in AP13×Vs16 full-sib population grown at two Oklahoma locations detected using composite interval mapping

SL	QTL	Chr.	Map position (cM)	LOD	PVE (%)	Additivity	Location-year
1	FL_2K_50482208	2K	91.60	2.76	12.80	−2.54	Ard2008
2	FL_2K_22821656	2K	101.94	4.45	20.73	−3.18	Ard2008
3	FL_2K_7303895[a]	2K	134.62	3.67	17.03	−2.95	Ard2008
4	FL_3K_8820134	3K	24.74	3.49	16.25	2.70	Ard2008
5	FL_3K_10580541	3K	27.66	3.49	14.85	2.79	Ard2008
6	FL_3K_10880925	3K	31.48	5.15	24.08	3.56	Ard2008
7	FL_3K_11371679[a]	3K	35.54	5.78	26.95	3.69	Ard2008
8	FL_5N_86034691[a]	5N	30.94	5.38	25.12	3.53	Ard2008
9	FL_7K_12994979[a]	7K	123.82	3.42	15.82	−2.81	Ard2008
10	FL_1K_4716538[a]	1K	36.18	3.35	15.37	7.58	Ard2009
11	FL_6N_14823958	6N	111.72	2.50	11.31	−4.39	Ard2009
12	FL_7N_2939277[a]	7N	57.63	4.45	20.51	−6.12	Ard2009
13	FL_7N_8389391	7N	62.63	3.21	15.17	−5.42	Ard2009
14	FL_7N_3756112	7N	64.81	3.82	17.59	−5.84	Ard2009
15	FL_8N_39745296	8N	49.98	2.58	12.02	−4.77	Ard2009
16	FL_1N_35092595	1N	74.14	4.38	20.41	−4.30	Ard2010
17	FL_1K_41694291	1K	68.11	5.42	25.56	−4.21	Ard2010
18	FL_1K_55863262[a]	1K	76.61	4.50	20.81	−3.65	Ard2010
19	FL_2N_71016516	2N	98.37	2.64	12.27	−2.93	Ard2010
20	FL_2N_101555261[a]	2N	166.01	4.27	19.79	3.63	Ard2010
21	FL_3K_26785266	3K	77.20	2.93	13.53	−2.92	Ard2010
22	FL_3K_44093513	3K	84.38	3.49	16.13	−3.17	Ard2010
23	FL_3K_43676042	3K	87.39	2.84	13.49	2.86	Ard2010
24	FL_1N_7312478	1N	129.81	5.05	23.37	−3.45	Ard2011
25	FL_1N_6206723[a]	1N	135.98	5.82	26.79	3.49	Ard2011
26	FL_1K_60899297[a]	1K	86.93	4.39	20.26	−3.01	Ard2011
27	FL_1K_64692294	1K	94.65	3.21	14.84	−2.54	Ard2011
28	FL_6N_18085264[a]	6N	116.38	4.21	19.49	−3.01	Ard2011
29	FL_7N_46914472[a]	7N	42.48	4.67	21.73	−3.21	Ard2011
30	FL_4K_48108041	4K	86.25	2.50	11.37	−3.83	RR2009
31	FL_6K_49650038	6K	50.45	3.73	17.38	7.05	RR2009
32	FL_5K_56908841	5K	66.01	2.51	11.64	2.04	RR2010
33	FL_7N_48170140	8N	65.87	2.91	13.44	2.23	RR2010
34	FL_2K_326829	2K	144.70	3.64	17.05	−4.54	RR2011
35	FL_3K_41190937	3K	90.17	4.18	19.28	4.64	RR2011
36	FL_3K_49098567	3K	95.79	3.15	14.97	4.08	RR2011
37	FL_9N_55219699	9N	54.12	2.75	12.84	−4.00	RR2011

Note. Chr.: chromosome; LOD: logarithm of odds; PVE: phenotypic variance explained.
[a]QTL annotated.

the genes associated with QTL controlling a trait (Zou et al., 2012). Complex genetic bases of quantitative traits, accompanied by strong environmental influences, affect QTL locations and estimation of their effects (Robertson-Hoyt et al., 2006). Increase of number of markers and population size and integration of data from multiple environments overcomes some of these limitations ((Robertson, Payne, & Holland, 2006). QTL and associated candidate genes for corn *Fusarium* air rot disease were identified after integration of GBS SNPs in linkage maps (Maschietto et al., 2017).

TABLE 6 QTL for vegetative growth period in AP13×VS16 full-sib population grown at two Oklahoma locations detected using composite interval mapping

SL	QTL	Chr.	Map position (cM)	LOD	PVE (%)	Additivity	Location-Year
1	VP_2K_43261176	2K	62.98	3.13	14.55	2.84	Ard2008
2	VP_2K_31522257	2K	68.52	4.39	20.22	−3.51	Ard2008
3	VP_2K_48567403	2K	70.09	3.13	14.45	−2.90	Ard2008
4	VP_2K_7303895[a]	2K	132.61	3.39	15.65	−2.88	Ard2008
5	VP_3K_68774945[a]	3K	140.99	4.11	19.00	3.75	Ard2008
6	VP_5N_78476927	5N	42.97	4.54	21.09	3.35	Ard2008
7	VP_7K_12994979	7K	123.82	2.57	11.91	−2.55	Ard2008
8	VP_1K_62780264	1K	92.11	2.25	11.66	−4.17	Ard2009
9	VP_3K_3071522	3K	4.85	3.28	15.23	5.29	Ard2009
10	VP_8N_40657439	8N	44.96	4.35	20.22	−16.01	Ard2009
11	VP_9N_63132454[a]	9N	49.00	3.50	16.15	5.46	Ard2009
12	VP_1N_2692738	1N	137.98	2.50	11.49	−4.28	Ard2010
13	VP_1N_971474	1N	149.35	2.83	13.10	3.26	Ard2010
14	VP_1K_60899297	1K	86.93	3.25	15.02	−3.15	Ard2010
15	VP_1K_64937776	1K	96.55	3.67	17.14	−3.66	Ard2010
16	VP_2N_100383441	2N	166.71	3.16	14.73	3.51	Ard2010
17	VP_1N_7312478	1N	129.81	3.44	16.23	−2.68	Ard2011
18	VP_1N_6206723[a]	1N	135.98	4.44	20.46	2.86	Ard2011
19	VP_1K_57091180[a]	1K	79.46	3.58	16.46	−2.62	Ard2011
20	VP_3K_8359550	3K	20.76	3.24	15.10	2.55	Ard2011
21	VP_9N_53557827[a]	9N	47.99	3.04	14.07	2.34	Ard2011
22	VP_1N_37630527	1N	83.70	2.79	12.95	−4.52	RR2009
23	VP_5K_8876804	5K	79.98	3.00	13.83	4.72	RR2009
24	VP_5N_5423797	5N	148.32	2.78	12.81	−4.14	RR2009
25	VP_5N_2306386	5N	152.51	2.91	13.42	−4.21	RR2009
26	VP_5N_662236	5N	157.52	2.99	13.68	−4.24	RR2009
27	VP_1N_91058452	1N	15.71	2.54	11.68	−5.37	RR2010
28	VP_1N_80838866	1N	22.52	2.51	11.68	−5.38	RR2010
29	VP_3K_6794064	3K	17.89	3.68	16.90	−2.97	RR2010
30	VP_2K_72839119[a]	2K	18.69	3.81	17.70	−5.75	RR2011
31	VP_2K_67003274	2K	26.23	4.95	22.96	6.38	RR2011
32	VP_2K_66770667[a]	2K	29.08	4.81	22.28	−6.34	RR2011
33	VP_3K_3071522	2K	4.85	2.71	12.58	4.45	RR2011
34	VP_9N_70062036	9N	62.96	4.96	23.10	−6.70	RR2011

Note. Chr.: chromosome; LOD: logarithm of odds; PVE: phenotypic variance explained.
[a]QTL annotated.

Several linkage maps were reported in switchgrass using RFLP (Missaoui, 2003); SRDF (Missaoui et al., 2005); SSR (Liu, Wu, Wang, & Samuels, 2012; Okada et al., 2010; Serbaet al., 2015, 2013; Wang, Samuels, & Wu, 2011); STS (Okada et al., 2010; Serba et al., 2013); DArT (Serba et al., 2013); and SNP (Lu et al., 2013) markers. However, none of the map had a reasonably good marker coverage. Thus, the linkage map generated in this study, which was aligned with the published switchgrass genome, shows great importance for future endeavor in QTL mapping and application of MAS. Currently, a small obstacle in using such map for QTL mapping is the limitation of marker numbers in chromosomes (Maschietto et al., 2017; Serbaet al., 2015, 2013). Thus, reduction of marker number in each chromosome was done systematically, taking uniform marker distribution into account, for this QTL mapping using QTL cartographer.

TABLE 7 QTL detected from BLUP (best linear unbiased prediction) values estimated across all environments for spring green-up, days to flower, and vegetative growth period using composite interval mapping in the AP13×VS16 full-sib population

SL	QTL/marker	Chr.	Map position (cM)	LOD	PVE (%)	Additivity
1	SG_BLUP_1N_12597421	1N	121.01	2.73	12.65	−0.64
2	SG_BLUP_5K_114081764	5K	155.73	2.50	11.36	0.57
3	SG_BLUP_6N_22131630	6N	47.09	2.70	12.46	0.75
4	SG_BLUP_6N_17266366	6N	106.64	3.92	18.14	−0.85
5	SG_BLUP_9N_57708372	9N	30.50	4.43	20.33	−0.78
6	SG_BLUP_9N_28115531	9N	34.08	3.70	17.24	−0.73
7	FL_BLUP_1K_54246796	1K	85.37	5.74	26.76	−4.06
8	FL_BLUP_1K_64692294	1K	94.65	9.45	43.65	−6.17
9	FL_BLUP_1K_70369866	1K	100.78	3.77	17.46	−1.93
10	FL_BLUP_1K_71330793	1K	105.35	5.43	25.29	4.09
11	FL_BLUP_2N_23594225	2N	76.31	3.45	15.44	−1.69
12	FL_BLUP_3K_14668834	3K	46.40	2.67	12.37	1.57
13	FL_BLUP_7K_10119976	7K	119.49	2.50	11.49	−1.38
14	VP_BLUP_1K_57091180	1K	79.46	8.07	37.06	−4.24
15	VP_BLUP_2N_23594225	2N	76.31	2.82	13.07	−1.66
16	VP_BLUP_3K_14668834	3K	46.40	2.65	12.26	1.62

Note. Chr.: chromosome; LOD: logarithm of odds; PVE: phenotypic variance explained.

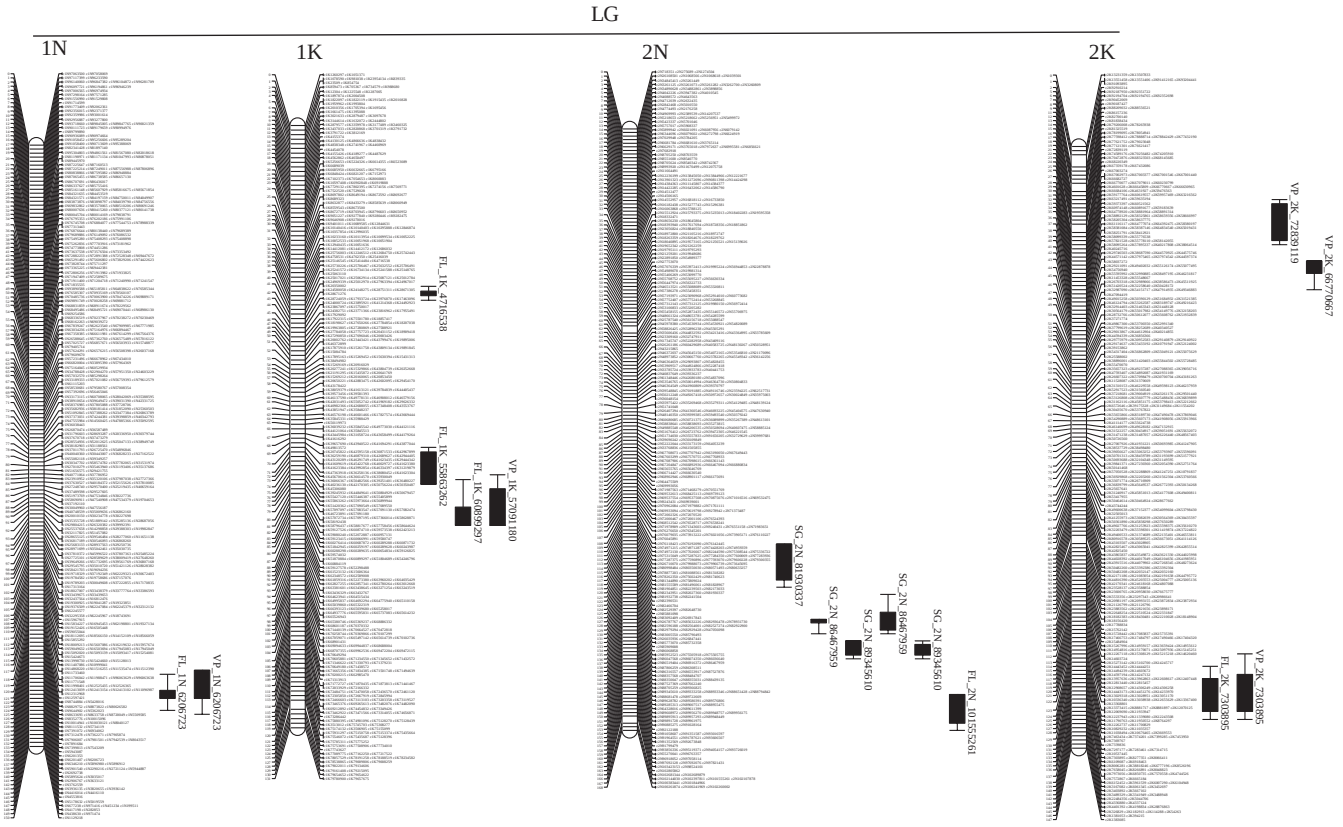
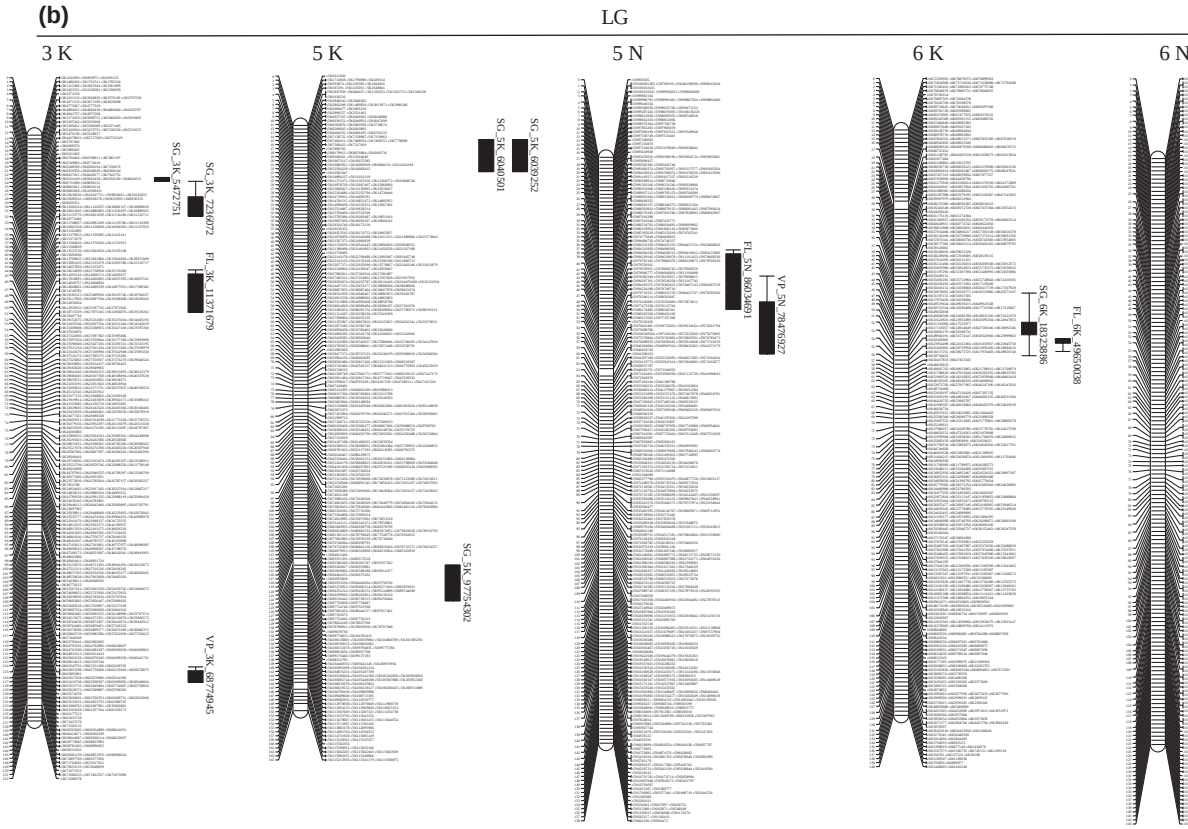


FIGURE 6 Distribution of spring green-up (SG), flowering (FL), and vegetative growth period (VG) QTL on switchgrass chromosomes. The solid bars represent QTL location, and the whisker on one or both ends of the QTL chart indicates LOD scores ≥1.0 but <2.5



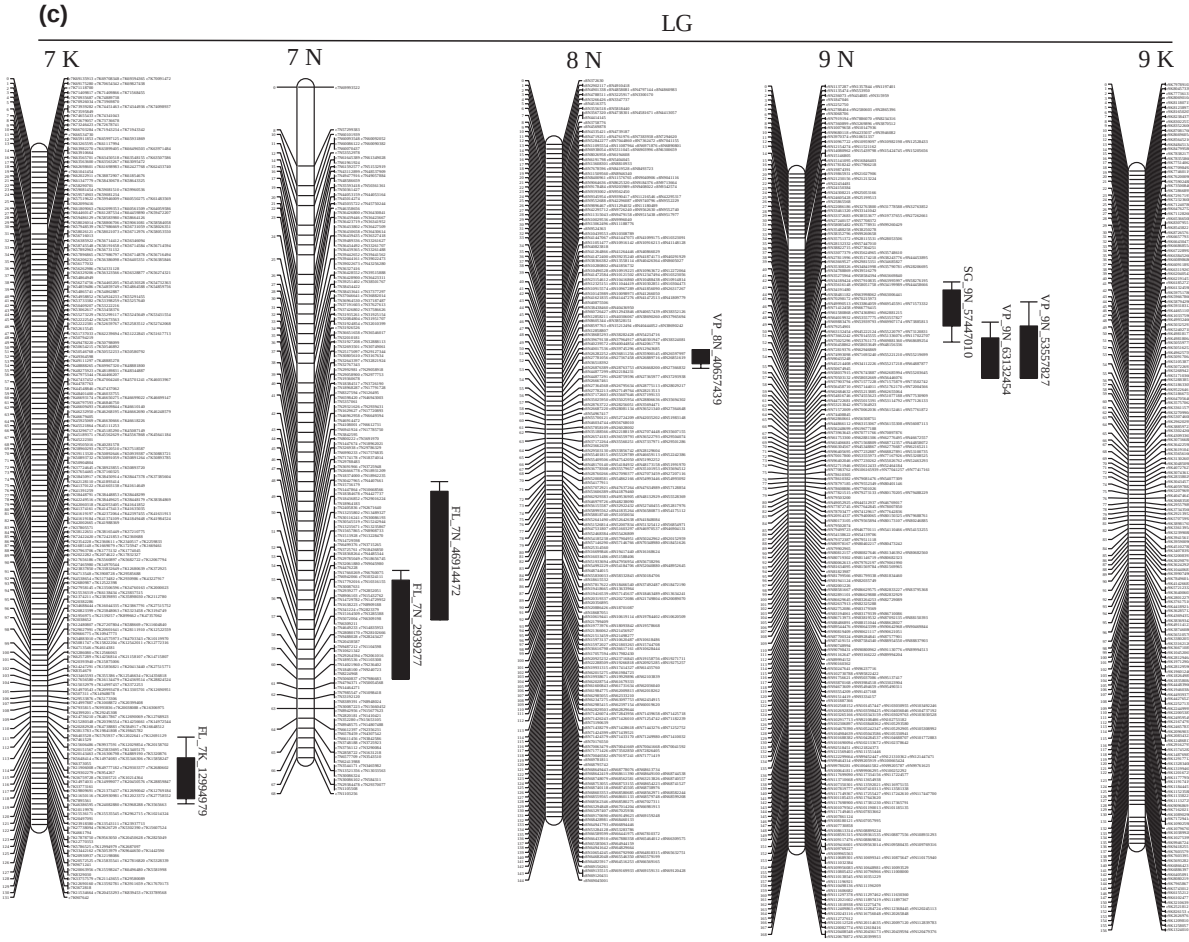


FIGURE 6 (Continued)

TABLE 8 List of candidate genes flanking 50 kb upstream and downstream of the peak markers of the major QTLs

QTL	Chromosome	Switchgrass gene ID and description	mRNA Start	mRNA End	Strand (±)	Functional categories
SG_2N_81930337 (Ard2008)	Chr02N	Pavir.2NG450200.1: Abscisic acid-responsive, HVA22, like protein	81881620	81882847	−	Hormone-mediated signalling pathway/ protein binding
	Chr02N	Pavir.2NG450200.2: Abscisic acid-responsive, HVA22, like protein	81881620	81882847	−	Hormone-mediated signaling pathway/ protein binding
	Chr02N	Pavir.2NG450300.1: Endoglucanase 10-related cellulase	81883267	81887642	−	Carbohydrate metabolic process/ hydrolase activity
	Chr02N	Pavir.2NG450300.2: Endoglucanase 10-related cellulase	81883564	81887654	−	Carbohydrate metabolic process/ hydrolase activity
SG_2N_86467959 (Ard2008)	Chr02N	Pavir.2NG478000.1: Protein kinase family protein	86512295	86516470	−	Protein phosphorylation/protein kinase activity
SG_2N_89345610 (Ard2008)	Chr02N	Pavir.2NG509700.1: WRKY transcription factor 24-related	89311562	89318353	−	Transcription regulation/DNA binding
	Chr02N	Pavir.2NG510000.1: Senescence regulator (Senescence, reg)	89335583	89336633	−	Transcription regulation/DNA binding
	Chr02N	Pavir.2NG510100.1: DHHC-type zinc finger domain-containing protein	89345577	89347909	−	Transcription regulation/DNA binding
	Chr02N	Pavir.2NG510100.2: DHHC-type zinc finger domain-containing protein	89345577	89347909	−	Transcription regulation/DNA binding
SG_5K_97754302 (Ard2008)	Chr05K	Pavir.5KG588200.1: PsbP domain-containing protein 6	97730837	97732312	+	Photosynthesis
	Chr05K	Pavir.5KG588300.1: UDP-glucosyl/glucuronosyl transferase	97761668	97764745	+	Metabolic process/transferase activity
SG_9N_57447010 (Ard2008)	Chr09N	Pavir.9NG384300.1: No apical meristem (NAM) protein	57484915	57487851	+	Transcription regulation/DNA binding
SG_3K_5472751 (Ard2010)	Chr03K	Pavir.3KG063000.1: MADS box transcription factor protein/AGAMOUS-like 12	5431024	5436487	−	Transcription regulation/DNA binding
SG_3K_7236072 (Ard2010)	Chr03K	Pavir.3KG085500.1: Endo-1,4-beta-glucanase/glycosyl hydrolase 9C2	7188144	7190930	+	Carbohydrate metabolic process/ hydrolase activity

(Continues)

TABLE 8 (Continued)

QTL	Chromosome	Switchgrass gene ID and description	mRNA Start	mRNA End	Strand (±)	Functional categories
FL_3K_8820134 (Ard2008)	Chr03K	Pavir.3KG097900.1: Cytochrome B5 isoform B	8824271	8827285	+	Oxidation–reduction process/heme binding
	Chr03K	Pavir.3KG098200.1: Beta-sesquiphellandrene synthase/ terpene synthase1 (Tps1)	8855955	8859293	+	Metabolic process/terpene synthase activity
	Chr03K	Pavir.3KG098400.1: Beta-sesquiphellandrene synthase/ terpene synthase1 (Tps1)	8870023	8873401	−	Metabolic process/terpene synthase activity
	Chr03K	Pavir.3KG098400.2: Beta-sesquiphellandrene synthase/ terpene synthase1 (Tps1)	8870023	8873401	−	Metabolic process/terpene synthase activity
	Chr03K	Pavir.3KG098400.3: Beta-sesquiphellandrene synthase/ terpene synthase1 (Tps1)	8870023	8873401	−	Metabolic process/terpene synthase activity
	Chr03K	Pavir.3KG098400.4: Beta-sesquiphellandrene synthase/ terpene synthase1 (Tps1)	8870023	8873401	−	Metabolic process/terpene synthase activity
FL_3K_11371679 (Ard2008)	Chr03K	Pavir.3KG129700.1: MYB108 transcription factor protein	11376863	11378927	−	Transcription regulation/DNA binding
FL_7K_12994979 (Ard2008)	Chr07K	Pavir.7KG051800.1: Flavonoid 3′,5′-hydroxylase (F3′5′H)/ Cytochrome P450	12346191	12350395	+	Oxidation–reduction process/heme binding
FL_7N_46914472 (Ard2011)	Chr07N	Pavir.7NG181000.1: Ethylene-insensitive protein 3 (EIN3)	46962736	46964693	+	Transcription regulator/DNA binding
VP_2K_7303895 (Ard2008)	Chr02K	Pavir.2KG061400.1: CXC domain-containing protein, TESMIN/TSO1-related	7349963	7355277	+	Transcription regulator/DNA binding
	Chr02K	Pavir.2KG061400.4: CXC domain-containing protein, TESMIN/TSO1-related	7350913	7355138	+	Transcription regulator/DNA binding
	Chr02K	Pavir.2KG061400.3: CXC domain-containing protein, TESMIN/TSO1-related	7350878	7355138	+	Transcription regulator/DNA binding
	Chr02K	Pavir.2KG061400.2: CXC domain-containing protein, TESMIN/TSO1-related	7350666	7355139	+	Transcription regulator/DNA binding
VP_1K_57091180 (Ard2011)	Chr01K	Pavir.1KG323300.1: digalactosyldiacylglycerol synthase/ UDP-Glycosyltransferase	57060894	57065253	+	Carbohydrate metabolic process/ hydrolase activity
	Chr01K	Pavir.1KG323600.1: VQ motif (VQ)	57104209	57105828	+	Unknown

Note. Ard2008: observed at Ardmore in 2008; Ard2011: observed at Ardmore in 2011; FL: days to flowering; SG: spring green-up; VP: vegetative growth period.

QTL for these growth traits were mapped on all of the 18 chromosomes although some of these possess more QTL than others. The variability of QTL identification between the two locations might be due to the effect of environmental variations. A drought period prevails in 2011 in Oklahoma during the field evaluation of the progenies (Serba et al., 2015). Although late rainfall at the RR location did not affect biomass yield, the drought might have affected FL and VP, assuming that SG escaped the drought. It should be noted that RR has sandy soil, whereas Ardmore soil is clay-loam. Variations of soil type in conjunction with the drought in the growing season might influence the expression of growth traits. Detection of environmentally responsive QTL was reported earlier for flowering and plant height in rice (Li et al., 2003), for regrowth after harvest in sorghum (Murray et al., 2008), for flowering QTL in barley (Cuesta-Marcos et al., 2008), and for biomass yield and plant height in switchgrass (Serba et al., 2015).

Identification of pleiotropic QTL that controls more than one trait and markers linked to these QTL can be used to simultaneously select for two or more traits, which would have a practical implications in plant breeding. In this study, high correlation coefficients between FL and VP were identified, and the colocalization of several QTL for these two traits indicates that simultaneous selection of more than one growth traits is possible to genetically improve switchgrass for biomass production.

Mapping of the major QTL revealed 11 genomic regions controlling the three growth traits. These genomic regions possessed at least two major QTL. SG QTL localized on genomic regions of chromosomes 2N, 3K, and 5K; FL QTL on 1K and 7N; VP QTL on 2K and 9N. Serba et al. (2015) reported the presence of major genomic regions controlling switchgrass biomass yields on chromosomes IIb (2K), IIIa (3K), VIa (6K), IXa (9N), and IXb (9K). The FL QTL are also in agreement with reproductive maturity QTL identified on 1N, 2N, 3K, and 7K (Dong et al., 2015).

Annotation of genes flanking the peak markers associated with 30 major QTL revealed a number of genes that are involved in plant growth and development, flowering, and important biochemical processes. Among these genes, *REG*, *HVA22* (ABA- and stress-inducible gene), *PspB*, *NAM*, and *WRKY14* were found associated with SG QTL (Table 8). Manipulation of REG and *HVA22* genes increases biomass production by keeping leaves photosynthetically active and inhibiting hormone-mediated programmed cell death in plants (Gan & Amasino, 1995; Guo & Ho, 2008; Nelson, 1988). Knocking out of *PspB*, a key gene in plant photosystem II, resulted in a too drastic reduction of plant growth (Ifuku, Yamamoto, Ono, Ishihara, & Sato, 2005). *NAM* homolog was an immediate target of floral homeotic gene, *AP3/PI* (*APETALA3/PISTILLATA*), which controls flower development (Sablowski & Meyerowitz, 1998). *WRKY* proteins have important roles in a broad range of biological processes in plants including plant hormone signaling (Chen et al., 2010; Shang et al., 2010), plant growth and development (Johnson, Kolevski, & Smyth, 2002; Luo, Dennis, Berger, Peacock, & Chaudhury, 2005), and leaf senescence (Miao, Laun, Zimmermann, & Zentgraf, 2004; Robatzek & Somssich, 2002).

MYB108 and EIN3 were found associated with FL QTL. *MYB* type transcription factor genes regulate number of plant growth and developmental processes. In association with *MYB24, MYB108* regulates the jasmonic acid-mediated stamen maturation (Mandaokar, 2009). *EIN3* mutants showed cell growth inhibition and accelerated senescence in Arabidopsis (Chao et al., 1997). *TSO1* (CXC domain-containing protein) and *VQ* (valine-glutamine repeat) motif protein were found associated with VP QTL. *TSO1* was required for both male and female fertility, and *TSO1* mutant caused defects in flower and ovule development (Andersen et al., 2007; Hauser, He, Park, & Gasser, 2000). *VQ* proteins play an important role in plant growth and development by acting as cofactor of *WRKY* transcription factors (Cheng et al., 2012). The findings of this research indicate that biomass yield can be improved by manipulating these genes through breeding or molecular approaches. Some of these genes controls multiple developmental stages of plants. Nevertheless, this finding is a step forward in implementing MAS in breeding for biomass yield improvement in switchgrass.

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