

HIGH-DENSITY GENETIC MAP CONSTRUCTION AND QTL MAPPING IN THE SORGHUM-SUDANGRASS RIL POPULATION (M81×Sa)

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Abstract

Sorghum-sudangrass, a hybrid of sorghum and sudangrass has high productivity, strong adaptability, and superior quality and is widely used in animal husbandry and aquaculture. Parsing the genetic basis of major agronomic traits is very important for sorghum and sudangrass hybrid breeding. This study used a sweet sorghum M81 and sudangrass Sa hybrid to construct 174 F₁₁ populations. Single-nucleotide polymorphisms (SNPs) derived from genotyping using its Restriction-site Associated DNA Sequence (RAD-seq) of RILs to construct a high-density genetic map comprising 790 SNPs spanning 2101 cM, in which coverage of all ten chromosomes was greater than 80%. Inclusive Composite Interval Mapping (ICIM) was carried out to map Quantitative trait loci (QTL) for five forage traits. A total of 18 QTLs were identified for plant height (PH, 4 QTLs), fresh weight (FW, 8 QTLs), dry weight (DW, 3 QTLs), stem diameter (SD, 2 QTLs), and sugar content (SC, 1 QTL). In addition, two overlapping QTLs (*qFW9.2/qDW9* and *qFW10/qDW10.2*) were detected for FW and DW. Together, the RIL populations and high-density linkage maps in this study provide a theoretical basis for future sorghum-sudangrass hybrid forage breeding.

Key words: Sorghum-sudangrass; RAD-seq; Genetic map; QTLs

Introduction

Sorghum (*Sorghum bicolor* Moench) is considered the world's fifth largest food crop species (Smith *et al.*, 1986). Sweet sorghum is a member of the sorghum genus (Naoura *et al.*, 2020), and both sudangrass and sweet sorghum are crop species of the Gramineae family. The chromosomal arrangements of both species are 2n=20. Compared with sorghum, hybrids of sorghum and sudangrass constitute an annual forage crop with many advantageous characteristics, such as high yield, good nutritional quality, drought resistance, lodging resistance, and cutting resistance.

Recent research on Quantitative trait loci (QTLs) in sorghum has been extensive and focused on those governing the flowering period, drought resistance, disease resistance, crop yield, estimated juice weight, PH, and SD (stem diameter) (Crasta *et al.*, 1999; Wu *et al.*, 2007; Shiringani *et al.*, 2010; Yousra *et al.*, 2012). However, there are relatively few research reports on QTL mapping in sudangrass. Lu *et al.*, (2011) selected F_{2:3} population as test materials, used 170 AFLP (Amplified Fragment Length Polymorphism) and 8 RAPD (Randomly Amplified Polymorphic DNA) to construct a linkage map, located 4 QTLs related to the number of leaves; 3 QTLs for yield per plant; 2 QTLs for plant height; 3 QTLs for tiller number; 2 QTLs for dry weight per plant; 5 QTLs for leaf length and width; 4 QTLs for the stem/leaf ratio and 3 QTLs for stem diameter. Sorghum sudanense and Sorghum-sudangrass hybrid F₂ populations were used to construct a high-density genetic linkage map of Sorghum-sudangrass, a total 24 QTLs were detected, and the number of QTLs controlling hydrocyanic acid content, leaf number, tiller number, dry weight per plant, stem diameter, leaf length, leaf width, panicle length and stem-leaf ratio, and plant height were 2,1,1,1,3,3,3,3,3 and 4, respectively (Fang *et al.*, 2015).

RAD-seq (Restriction-site Associated DNA Sequencing) has excellent advantages in research without

a reference genome and can obtain much genome variation information. Compared with the features of traditional molecular markers, the main feature of RAD-seq is its combination of two molecular biology techniques, which greatly improves the marker density (Hiromi *et al.*, 2020). Consequently, this technology has become increasingly popular in genetic mapping and QTL analysis. In sorghum, a high-density genetic map was constructed using RAD-seq from the RIL (recombinant inbred line) population detected 43 QTLs for 10 agronomic characters: 3 QTLs related to PH, 8 for SD, 10 for TN, 10 for FW, and 12 for DW (Jin *et al.*, 2021). In soybean, 78 QTLs for lead chlorophyll-content traits were identified (Wang *et al.*, 2020).

Here, 174 RILs of F₁₁ hybrids from a cross between sweet sorghum, M81, and sudangrass, Sa, were used to conduct the RAD-seq and construct the genetic map. Phenotypic analyses followed by QTL mapping indicated that five important forage quality traits can be mapped.

Material and Method

Study materials and growth conditions: A total of 174 RILs of F₁₁ hybrids from an M81 and Sa cross were studied. The RILs population were planted along with the two parents at an experimental field of the Anhui Science and Technology University (Fengyang, China) in 2019. A randomized complete block design was used with three replications. The field was managed under normal sorghum production conditions.

Phenotypic measurements and statistical analysis: Determination of agronomic traits, plant height (PH), dry weight (DW), sugar content (SC), stem diameter (SD), and fresh weight (FW), determination methods refer to Jin *et al.*, (2021). Three biological replicates were used for each phenotype measurement.

The frequency distribution and statistical analysis of phenotypic measurement were performed using GraphPad Prism 8. The coefficient of variation (CV), correlation coefficient, and analysis of variance (ANOVA; $p < 0.05$ and $p < 0.01$) of the phenotypes in the mapping population were analyzed with PROC GLM in SAS 9.4 (SAS Institute, Cary, NC, USA).

DNA extraction and sequencing: Sorghum young leaves from RILs and two parents were collected and stored on liquid nitrogen and then ground with a high-throughput plant tissue grinder (SPEX SamplePrep) for DNA extraction using the DNA secure Plant Kit. All DNA samples were normalized, and a library was constructed according to the reference by Jin *et al.*, (2021). Genotyping was performed using BGISEQ-2000 (Shenzhen, China).

SNP identification: Fseq software filtered and analyzed the SNP (single-nucleotide polymorphism) sequencing data obtained by RAD data analysis to obtain high-quality sequencing reads. Tassel software was used to extract SNP markers and filter high-quality SNP loci. Then BWA MEM (http://hannonlab.cshl.edu/fastx_toolkit/) was used to map filtered clean reads back to the reference genome BTx623 V3.1.1. The parameters detected variation and imputation default parameter references (Li *et al.*, 2009; Mckenna *et al.*, 2010).

Genetic map construction and QTL mapping: ICIMapping (Inclusive Composite Interval Mapping) was used to construct the genetic linkage map and QTL mapping, and gene effect analysis was carried out by QTL ICIMapping software with a significant threshold value for QTL at a LOD (logarithm of the odds) score of 2.5. The QTL mapping software was used to detect QTLs for each trait (Jin *et al.*, 2021).

Results

Construction of high-density linkage mapping: Of the total of 3461 SNP markers for RILs, 2671 markers did not conform to the Mendelian separation ratio; the partial separation ratio was 77.17%. After filtering and removal, markers were available for 790 SNPs. A high-density linkage map of the sorghum-sudangrass hybrid was constructed by 790 SNP makers and covered 2,101 cM of the genome (Fig. 1), with an average marker density of 2.65 cM. Among the ten linkage groups, LG10 contains the most markers with an average distance of 1.5 cM but with 31.8 cM and 31.6 cM gaps. The other markers are relatively dense, while LG1 contains the least markers, with an average marker spacing of 3.49 cM but an overall uniform distribution with no gap larger than 20 cM (Fig. 1 and Table 1).

According to the comparative analysis of the sorghum chromosome genome size, the overall coverage of all ten chromosomes was greater than 80%. Specifically, the coverage of chromosomes 2, 4, 6, 7, and 9 was greater than 90%; the coverage of chromosomes 1, 8, and 10 was greater than 85%; and that of chromosome 9 was nearly complete at 99.81% coverage. Chromosome 5 had the

lowest coverage, at 84.42%. These results indicated that the overall coverage of the ten chromosomes is relatively high and comprehensive.

Phenotype analysis: There were significant differences in all five attributes of M81 and Sa. There were statistically significant differences among the RIL population in FW, DW, PH, SC, and SD (Table 2). Compared to Sa, M81 had higher FW, DW, PH, SC, and SD (Table 2 and Fig. 2). Frequency distributions of PH, DW, PH, SC, and SD in the RILs were continuous and approximately normal (Fig. 2). Meanwhile, PH, DW, PH, SC, and SD had wide coefficients of variations (Table 2), indicating that these were quantitative traits controlled by multiple loci. As a result, this population was suitable for genetic analysis of these traits.

Person's correlation coefficients among the five traits were evaluated. A significant positive correlation between different traits was found, except for FW and SC, which had a positive correlation that was not significant (Table 3). Among them, the maximum correlation was between FW and DW, 0.79. The minimum correlation was between FW and SD and was 0.19 (Table 3). These results indicated that FW with DW, PH, and SD might be genetically linked.

Table 1. Characteristics of each linkage group.

Linkage group	Number of SNPs	Map distance (cM)	Average of distance (cM)	Mb	Coverage (%)
LG1	104	363	3.49	80.88	87.02
LG2	99	241	2.43	77.74	94.35
LG3	93	164	1.76	74.39	97.75
LG4	89	247	2.78	68.66	90.23
LG5	70	209	2.99	71.85	84.42
LG6	78	258	3.31	61.28	93.47
LG7	54	172	3.19	65.51	95.74
LG8	70	197	2.81	62.69	85.13
LG9	67	151	2.25	59.42	99.81
LG10	66	99	1.50	61.23	87.74
Total	790	2101	2.65	440.8	-

Table 2. Variation of five traits of parents and RILs population.

Type		Trait	DW	PH	SC	SD
		FW				
M81	Average	0.90	0.71	335.50	17.56	19.43
Sa	Average	0.45	0.36	321.33	7.08	12.39
RILs	Average	0.56	0.44	314.53	10.42	13.74
	SD	0.54	0.18	46.03	3.58	1.94
	Minimum	0.10	0.11	155.00	2.00	5.80
	Maximum	3.77	1.22	485.00	19.60	22.33
	CV %	74.89	47.06	16.62	39.77	16.89

1/: CV, the co-efficient of variation

Table 3. Correlation analysis of the RIL main agronomic traits.

Tarit	PH	SD	FW	DW
SD	0.43**			
FW	0.26**	0.19**		
DW	0.46**	0.34**	0.79**	
SC	0.34**	0.24**	0.07	0.33**

1/: * Means the 0.05 significance level

** Means the 0.01 extremely significance level

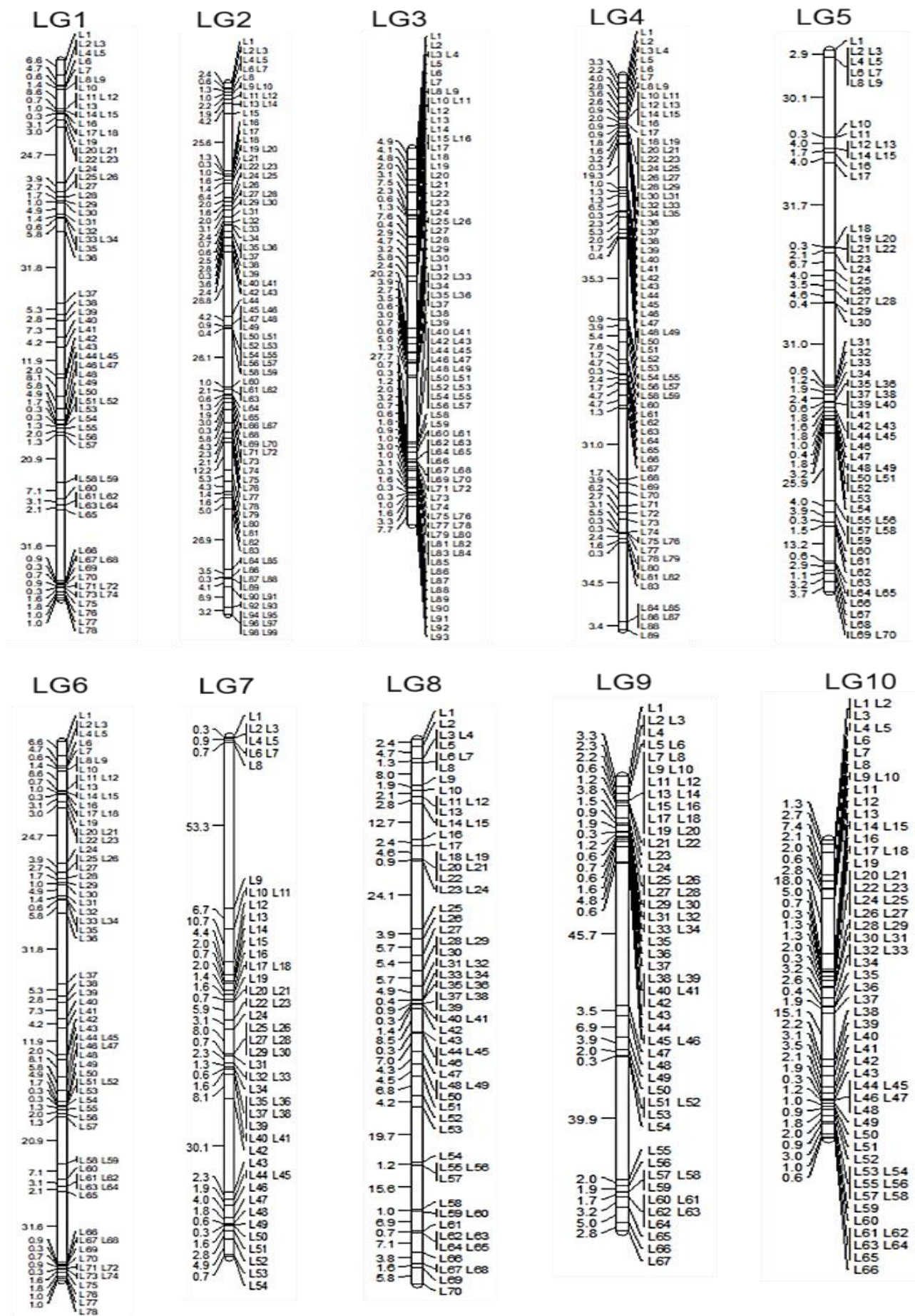


Fig. 1. A genetic linkage group of sorghum-sudangrass.

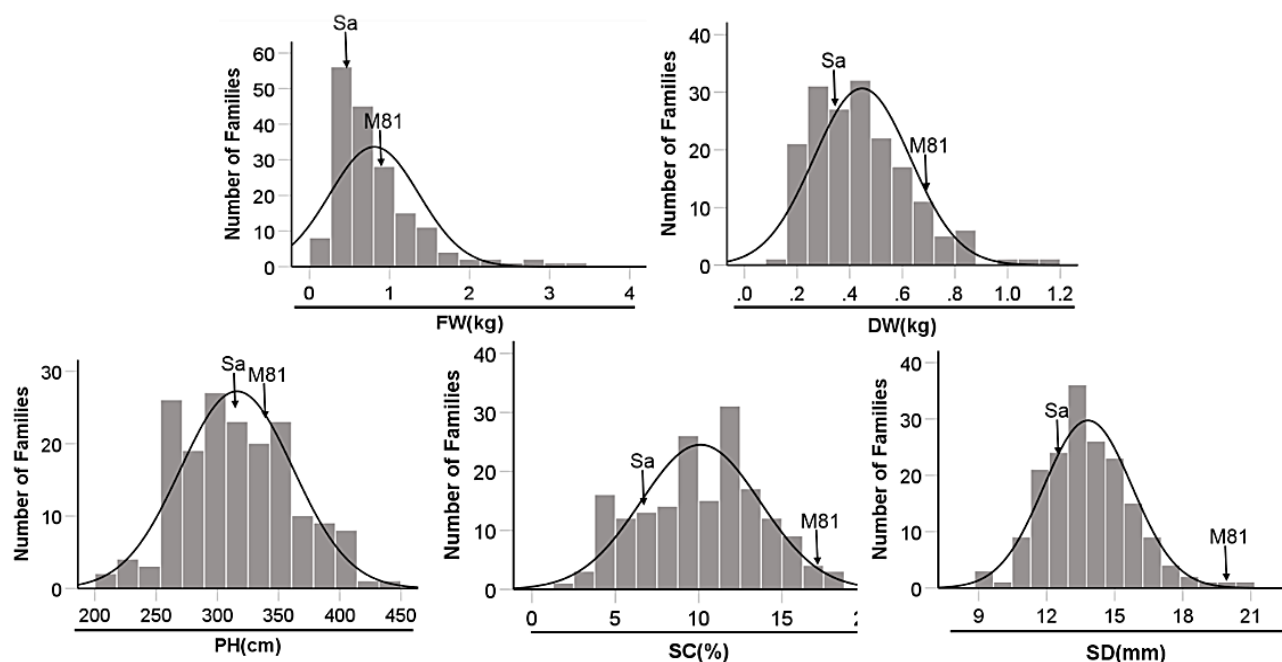


Fig. 2. Frequency distributions of PH, DW, PH, SC, and SD in the RILs population. Arrows indicate the phenotypic values of the parents.

Table 4. QTL analysis the agronomic traits in RILs.

Trait	QTL	LG	Marker interval(bp)	LOD	PVE (%)	ADD
FW	<i>qFW1</i>	1	9, 051, 997-61, 186, 017	10.70	3.58	0.88
	<i>qFW3</i>	3	3, 093, 166-71, 350, 579	8.65	3.77	0.73
	<i>qFW6</i>	6	48, 864, 475-59, 872, 066	12.06	3.68	-0.79
	<i>qFW7</i>	7	5, 827, 342-62, 681, 439	8.51	3.63	0.86
	<i>qFW9.1</i>	9	1, 840, 198-2, 863, 924	7.18	3.66	-0.8
	<i>qFW9.2</i>	9	2, 474, 919-8, 210, 663	5.26	2.21	-1.11
	<i>qFW9.3</i>	9	51, 072, 854-54, 196, 954	3.71	2.63	-0.91
	<i>qFW10</i>	10	14, 336, 762-57, 342, 524	12.55	3.42	0.79
DW	<i>qDW9</i>	9	2, 474, 919-8, 210, 663	3.37	4.56	-0.35
	<i>qDW10.1</i>	10	46, 953, 313-48, 084, 322	2.81	3.39	0.05
	<i>qDW10.2</i>	10	14, 336, 762-57, 342, 524	3.63	12.33	0.14
PH	<i>qPH3.1</i>	3	71, 014, 455-71, 350, 576	3.22	5.84	14.53
	<i>qPH3.2</i>	3	1, 321, 674-5, 663, 636	3.43	7.68	-24.53
	<i>qPH4</i>	4	56, 240, 496-57, 019, 648	3.90	7.03	15.2
	<i>qPH10</i>	10	44, 096, 611-46, 502, 920	2.66	4.41	12.8
SC	<i>qSC10</i>	10	55, 427, 127-59, 360, 047	2.68	5.43	-3.06
SD	<i>qSD7</i>	7	59, 963, 463-61, 231, 615	2.54	2.24	-0.55
	<i>qSD8</i>	8	2, 459, 915-48, 895, 979	2.79	6.59	-1.79

1/: The bold QTL indicates that control of multiple traits. PVE, Phenotypic variation explained. ADD, Additive effects

QTL analysis: QTLs were identified for FW, DW, PH, SD, and SC (Table 4). Eight FW QTLs were identified on chromosomes 1, 3, 6, 7, 9, and 10; the LOD value ranged from 3.71 to 12.55. *qFW10* displayed the highest LOD score (12.55). However, the phenotypic variations of these QTLs ranged from 2.21% to 3.77%, indicating that these QTLs are minor for FW. The total PVE (Phenotypic Variation Explained) is 26.58%. These FW QTLs from M81 (*qFW6*, *qFW9.1*, *qFW9.2*, and *qFW9.3*) showed additive and dominant effects, and the FW QTLs from Sa (*qFW1*, *qFW3*, *qFW7*, and *qFW10*) showed additive and dominant effects.

For the dry weight per plant (DW), three QTLs were identified for DW on chromosomes 9 and 10, with LOD

values from 2.81 to 3.63 and PVE values from 3.39% to 12.33%. Among them, *qDW10.2* had the highest PVE (12.33%). This DW QTL from M81 (*qDW9*) showed additive and dominant effects, as did these DW QTLs from Sa (*qDW10.1* and *qDW10.2*).

For plant height (PH), four PH QTLs were identified on chromosomes 3, 4 and 10, with LOD values from 2.66 to 3.90. The effect of increased PH was contributed to M81 at *qPH3.2*, and to the Sa allele at *qPH3.1*, *qPH4*, and *qPH10*.

For sugar content (SC), one QTL was identified and located on chromosome 10. Its LOD value was 2.68 explaining 2.24% of the phenotypic variation. This QTL effect of increased SC contributed to M81.

Table 5. Colocalization of QTLs mapping in this study with previously mapping.

Trait	This study		Previous study		
	QTL	Location	QTL	Location	Reference
FW	<i>qFW3</i>	3,093,166-71,350,579	<i>QTBMS</i>	1 6,162,031–7,030,484	(e.g. Hufnagel <i>et al.</i> , 2014)
	<i>qFW6</i>	48,864,475-59,872,066	<i>qFW6</i>	45,156,899–55,463,230	(e.g. Jin <i>et al.</i> , 2021)
<i>qFW9.3</i>	51,072,854-54,196,954		<i>QFBMS6.1</i>	47,686,626–50,991,177	(e.g. Wang <i>et al.</i> , 2014)
			<i>QFBMS6.2</i>	52,744,974–53,427,904	(e.g. Shiringani <i>et al.</i> , 2010)
			<i>qFW9.2</i>	47,561,074–54,947,044	(e.g. Jin <i>et al.</i> , 2021)
			<i>QFBMS9.13</i>	50,673,105–51,682,151	(e.g. Li <i>et al.</i> , 2016)
<i>qFW10</i>	14,336,762-57,342,524	<i>qFW10</i>	47,248,748–52,258,072	(e.g. Jin <i>et al.</i> , 2021)	
DW	<i>qDW10.2</i>	14,336,762-57,342,524	<i>QTDBM10.3</i>	56,715,021–61,226,724	(e.g. Felderhoff <i>et al.</i> , 2012)
SD	<i>qSD7</i>	59,963,463-61,231,615	<i>qFW10</i>	47,248,748–52,258,072	(e.g. Jin <i>et al.</i> , 2021)
			<i>qSD7.2</i>	58,885,433–62,720,932	(e.g. Jin <i>et al.</i> , 2021)
			<i>qBD7.1</i>	58,400,000–60,100,000	(e.g. Kong <i>et al.</i> , 2020)

For stem diameter (SD), two SD QTLs were identified on chromosomes 7 and 8. The LOD values were 2.54 and 2.79, and the PVE values were 2.24% and 6.59%, respectively. These two QTLs effect increased SD for M81.

Overlapping QTLs: This study found two overlapping QTLs: *qFW9.2/qDW9* and *qFW10/qDW10.2* (Table 4). The two QTL clusters were found on chromosomes 9 and 10 between 2,474,919-8,210,663 and 14,336,762-57,342,524, respectively. FW and DW also showed a significant positive correlation (Table 3).

Discussion

The rapid development of molecular biology and the wide application of high-throughput sequencing technology have promoted new breeding strategies to increase crop yield and improve important yield-related traits. Several genetic linkage maps have been created, and some progress has been made in the QTL mapping of related traits. However, most of them were constructed using restriction fragment length polymorphism (RFLP), or simple-sequence repeats (SSR), which present few markers and large QTL confidence intervals, limiting their use in QTL fine mapping and marker-assisted breeding (Liu *et al.*, 2015; Shi *et al.*, 2017; Yu *et al.*, 2018; Wang *et al.*, 2021). Compared with the above molecular markers, SNP markers are widely used in map construction and QTL mapping of a variety of crops due to their high density, uniform and extensive distribution on chromosomes, and high genetic stability (Smulders *et al.*, 2019; Zhang *et al.*, 2021). Our research constructed a high-density genetic map, which contained 790 SNPs markers, spanning 2101 cM, in which coverage of all ten chromosomes was greater than 80%.

Compared to rice and maize, the yield and yield-related traits of sudangrass were less reported than those in sorghum (Lu *et al.*, 2011; Jin *et al.*, 2021). In addition, there is no report about the sugar content in this hybrid. In this study, we constructed mapping for a sorghum and sudangrass hybrid population by RAD-seq and identified 17 QTLs associated with four forage traits. Six of these QTLs were mapped on the same region as in previous research are list in Table 5, demonstrating these QTLs are stable will be the focus of our attention in subsequent studies. Additionally, we identified 11 novel QTLs for four traits. Among them, *qDW10.2* contributed 12.33% of the

phenotypic variation and exhibited an additive effect (Table 4). In addition, we identified one QTL for sugar content in the hybrid population. The sugar content of sweet sorghum M81 was found to be significantly higher than sudangrass Sa (Table 2). Our study also revealed that *qSC10* contributed 5.43% of the phenotypic variation, and its increased sugar content was contributed to M81, but without any significant positive correlation with FW (Table 3). These results are consistent with previous research (Murray *et al.*, 2008), suggesting that these correlations in sorghum may limit indirect selection for sugar traits using morphological traits.

We analyzed the overlapping QTLs and found that two QTLs of FW and DW in the same region on chromosomes 9 and 10 (Table 4), respectively. That means these two QTLs have pleiotropy, which jointly affects FW and DW phenotypic characteristics. The overlapping QTLs have been reported in wheat and sorghum (Mace *et al.*, 2012; Lillemo *et al.*, 2013; Jin *et al.*, 2021). Our study showed that FW and DW have a significant positive correlation (Table 3). This may be the reason why FW and DW were co-located within the same region.

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