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QTL MAPPING OF FIVE FORAGE QUALITY TRAITS IN SORGHUM × SUDANGRASS

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Abstract

The quantitative trait locus (QTL) mapping for quality traits will improve the breeding of sorghum-sudangrass. In this research, we characterized the phenotype of 126 RILs and their parents –Tx623A (sorghum) and Sa(Sudangrass) for two successive years (2018 and 2019). The phenotypes included five forage quality traits which were crude protein (CP), neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) and the content of hemicellulose (HC). We also mapped 19 QTLs controlling these traits in a RIL population between sorghum Tx623A and sudangrass Sa. A high density genetic map was constructed by RAD-seq. There were 1065 markers and the total length was 1191.7 cM in the genetic map. Consequently, a total of 19 QTLs were detected for the five traits, which included one QTL for CP, five QTLs for NDF, three QTLs for ADF, two QTLs for ADL and eight QTLs for HC. Four overlapping QTLs were detected for NDF, ADF, ADL and HC. The research results will supply very meaningful information to make better the forage quality breeding in sorghum-sudangrass hybrids.

Key words: Forage quality traits, Genetic map, QTL, RAD-seq.

Introduction

Sorghum-sudangrass hybrid is regarded as a high-quality forage for animals. The forage inherited the advantages of its parents, the resistance to drought from sorghum and the high biomass yield from sudangrass (Zhan *et al*., 2008). Sorghum-sudangrass exhibits prominent inter-specific heterosis, especially in forage yield and quality (Liu *et al*., 2015). So, the genetic analysis for the hybrid will improve the breeding of sorghum-sudangrass and elucidate the genetic basis of inter-specific heterosis.

The major targets of forage breeding are to increase the forage digestibility and crude protein (CP) (Murray *et al*., 2008). Neutral detergent fiber (NDF), acid detergent fiber (ADF) and the content of hemicellulose (HC) were the main index to evaluate the forage digestibility. Murray *et al*. identified 7 QTLs for 5 forage quality traits in stem across three locations (Murray *et al*., 2008). 8 QTLs were identified for 5 forage quality traits from a population of 188 grain×sweet sorghum recombinant inbred lines (Shiringani & Friedt, 2011). Li *et al*. identified 12 QTLs for 5 forage quality traits by a F_2 populations (Li *et al*., 2015) , and they identified 42 SNPs associated with 5 forage quality traits using 245 sorghum accessions (Li *et al*., 2018). However, comparing to the researches on agronomic traits in sorghum, there were still few QTLs identified in forage quality traits. So, it limited the progress in forage quality improvement. In the study, we had 3 objectives: (i) to construct a high density genetic map of the recombinant inbred line(RIL) population from sorghum-sudangrass hybrid using RAD-seq (ii) to characterize the inheritance pattern and identify the controlling locus for 5 forage quality traits using a RILs population (iii) to provide important information for the improvement of the forage quality breeding in the hybrids of sorghum-sudangrass.

Materials and Methods

Plant materials and phenotype evaluation: 126 sorghum×sudangrass RILs were developed from a hybidization with Tx623A (sorghum, female parent) and Sa (Sudangrass, male parent). In May 2018 and 2019, the RILs and their parents were cultivated in the tested plots of Anhui Science and Technology University (Fengyang, China, 32°52' N, 177°33' E) The planting density was 50 cm×25 cm. It was applied normal agronomic practices through all the growth period.

After flowering, the upground parts of RIL plants and the parents' plants were hand-harvested. All the samples were dried in an oven at 75℃ for two days until their weight remained constant. All samples were pulverized by a fodder grinder and sifted by sieve of 40 mesh. Then, the samples were measured by near infrared (NIR) spectra with an Antaris™ II FT-NIR Analyzer (Thermo, USA). The model was used from previous research (Li *et al*., 2018) and their CP, NDF, ADF, ADL and HC contents were calculated with the model.

DNA extraction and sequencing: The leaves of RILs and parents were used to extract DNA using the DNAsecure Plant Kit (Qiagen, Cat.No. DP320). The library of parents and RILs used different way to constructing. For parents, a library of circularized DNA fragments of 200-400 bp was amplified to make a DNA nanoball (DNB). Then the library was sequenced on MGISEQ-2000 (Shenzhen, China). For RILs, all DNA samples of RILs were normalized to 20 ng/uL, and 10 μL of each sample was digested with two enzymes of PstI (CTGCAG) and MspI (CCGG) of at 37℃ for 2 h and then at 65℃ for 20 min using 10 μL each sample. These samples were ligated with adapters using T4 ligase (NEB) for 20 min. The ready samples were collected with the same volume and followed by PCR-amplification in a single tube to add illumina sequencing adapters. The PCR product concentration was quantified checked by a Qubit 3.0 fluorometer (Invitrogen). The GBS library was run on an Illumina Hiseq2500 (San Diego, CA, USA).

SNP calling: To get high quality reads, raw data was filtered using SOAPnuke (Chen *et al*., 2018) with the filter parameters '-n 0.01 -l 20 -q 0.3 -A 0.25 --cutAdaptor -Q 2 -G --polyX 50 --minLen 150'. Then, the clean reads from the sequencing machine were split into 126 individual files based on indexes, and filtered using fastx_barcode_splitter and fastq_quality_filter with parameters (-q 20 -p 80 -Q 33) of fastx_toolkit-0.0.13.2 (http://hannonlab.cshl.edu/fastx_toolkit/). The split sequencing data were mapped using the alignment algorithm BWA MEM (Li & Durbin, 2009). GATK was used to detect variation and call SNPs for RILs and parents(McKenna *et al*., 2010). SNPs were called with parameters 'QD<2.0, MQ<40.0, FS>60, SOR>3.0, MQRankSum<-12.5, ReadPosRankSum <-8.0'. Imputation was conducted with Beagle 5.0 with default parameter (Browning & Browning, 2007; Browning *et al*., 2018).

The construction of the genetic map: The polymorphic SNPs were selected and converted to A or B by the genotype of parents using Tassel 5.0(Bradbury *et al*., 2007). The reduancy SNPs were filtered and the high density genetic map was constructed withWindows QTL IciMapping version 4.0 (Meng *et al*., 2015).

The phenotype analysis and QTL mapping: Pearson's correlation coefficients and histograms were counted and drawn with R package PerformanceAnalytics. QTL mapping was constructed by Windows QTL IciMapping version 4.0 (Meng *et al*., 2015). Inclusive Composite Interval Mapping (ICIM) was used to verify the putative QTLs (Li *et al*., 2008). LOD>2.5 was the threshold to claim the exist of putative QTL. The putative QTLs were claimed with LOD>2.5 as the threshold

Results

The phenotype analysis: The mean phenotypic values for all traits evaluated in the RILs fell between the two parents which showed significant difference in all five quality forage traits (Table 1). Compared to Tx623A, Sa had higher NDF, ADF and HC and lower CP. The histogram of the five traits showed the normal distribution in 2018 and 2019 (Figs. 1A and B).

Pearson's correlation coefficients among the five traits were assessed. NDF was significantly and positively correlated with ADF, ADL and HC, but CP was not significantly correlated with the four traits in both years (Fig. 1). These results indicated that NDF, ADF, ADL and HC might be genetic linkage, or certain genes might play pleiotropic functions in dominating these phenotypes.

The genetic map construction and QTL mapping: There were 1065 markers in the genetic map as well as the total length was 1191.7 cM after the redundancy markers were filtered (Table 2 and Fig. 2). The shortest chromosome was Chr7 of 67.8 cM, yet the longest one almost three times to the shortest one was Chr2 of 185.9 cM. In the 10 chromosomes, it had the most markers on Chr2 which was 0.88 cM of the average markerdistance, whereas it had the least markers on Chr7 that was 1.25 cM of the average marker distance. The coverage in physical map ranged from 97.04% to 99.89%. The biggest coverage was on chromosome 1 and the least coverage was on chromosome 7 (Table 2). The results showed that the genetic map had high coverage for sorghum genome.

19 QTLs were identified for all five forage quality traits (Table 3). Crude protein (CP): Only one QTL was detected for CP on chromosomes 4 in 2019. The LOD scores and PVE values was 5.04 and 16.93%, respectively. In 2018, no QTL was detected for the trait. The difference of CP between two parents was very small. So, it was hard to detect QTLs.

Neutral detergent fiber (NDF): Five QTLs were detected for NDF on chromosomes 1, 4, 5, 7 and 8. The variation of phenotype was illustrated by every QTL arranged from 7.18 to 58.74. The maximum LOD score was 6.13, and the minimum LOD score was 2.53. Among these QTLs, the effect of increased NDF was attributed to Sa at qNDF1, qNDF7, qNDF8, but to the Tx623A alleles at qNDF4 and qNDF5. The qNDF4 was detected in both years.

Table 1. Variation of 5 forage quality traits of parents and RILs population.

Year	Type		Trait				
			CP	NDF	ADF	ADL	HC
2018	Tx623A	Average	6.60	57.42	33.11	4.69	24.31
	Sa	Average	6.10	61.39	35.84	4.55	25.55
	RIL	Average	6.30	57.59	33.15	3.46	24.44
		SD	0.0527	0.7259	0.4879	0.0697	0.2623
		Min	4.76	50.15	20.38	1.34	8.77
		Max	8.01	64.85	37.86	5.18	27.08
2019	Tx623A	Average	7.81	54.43	30.31	4.85	24.12
	Sa	Average	6.18	64.72	39.27	5.39	25.45
	RIL	Average	7.01	57.96	33.48	5.19	24.58
		SD	0.0489	0.2102	0.2145	0.0415	0.0934
		Min	5.91	52.70	27.27	3.83	20.90
		Max	8.77	63.47	38.45	6.36	26.69

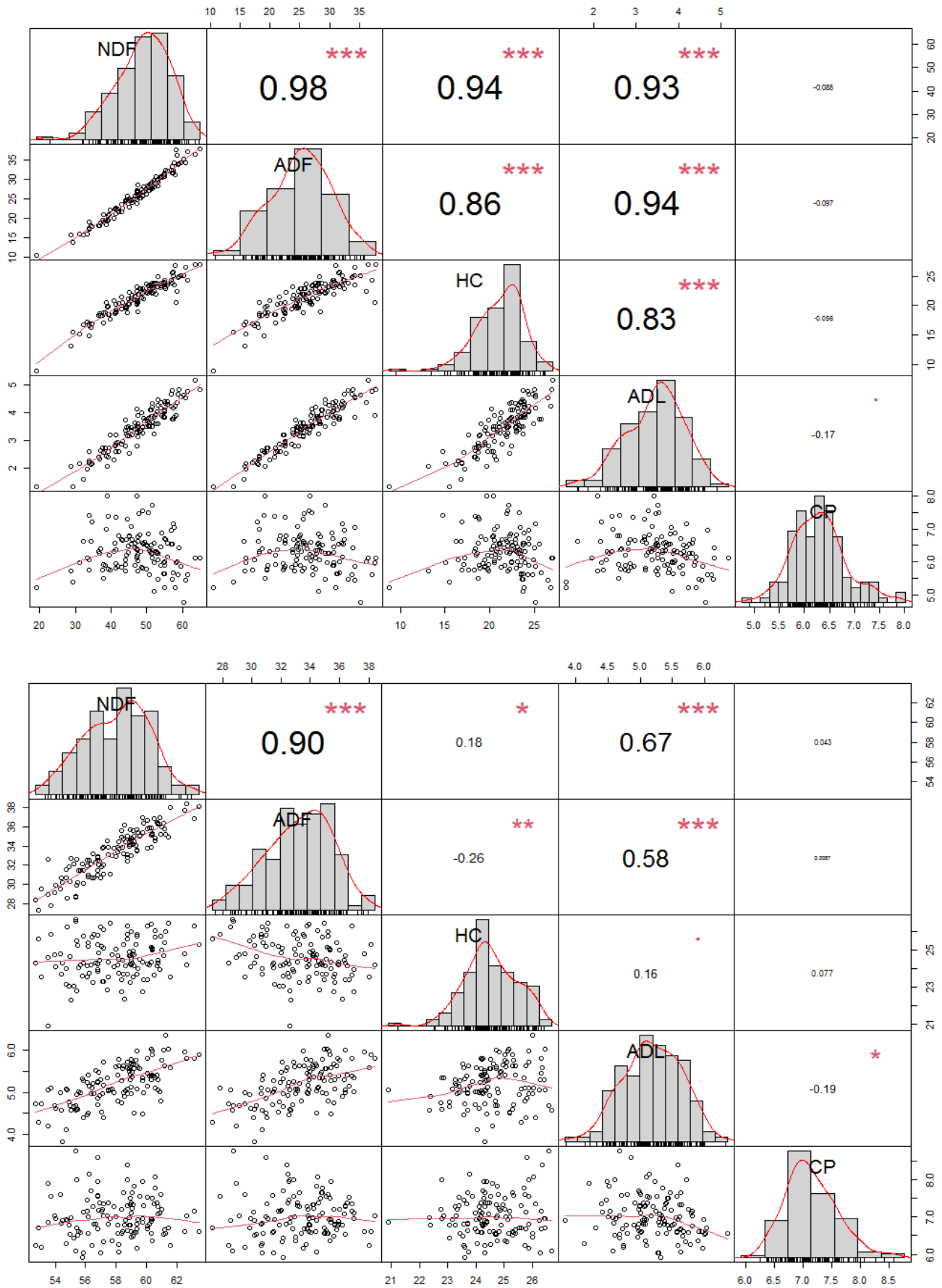

Fig. 1. The histogram and Pearson correlation coefficients for the 5 yield traits in two years. (A, the histogram and correlation analysis of the 5 traits in 2018; B, the histogram and correlation analysis of the 5 traits in 2019; * represents significantly at 0.05, ** represents significantly at 0.01, *** represents significantly at 0.001).

Table 2. RIL population's Statistical results of the genetic map.

Chr.	Marker number	Genetic length (cM)	Interval length (bp)	Physical map length (bp)	Coverage (%)
1	179	164.1	80,799,209	80,884,392	99.89%
2	209	185.9	77,387,954	77,742,459	99.54%
3	104	105.1	71,492,069	74,386,277	96.11%
4	117	131.6	68,011,155	68,658,214	99.06%
5	82	87.0	71,426,442	71,854,669	99.40%
6	67	101.5	59,720,942	61,277,060	97.46%
7	54	67.8	63,567,966	65,505,356	97.04%
8	94	127.6	61,413,726	62,686,529	97.97%
9	77	101.2	58,727,782	59,416,394	98.84%
10	82	119.9	60,810,786	61,233,695	99.31%
Total	1065	1191.7		-	
Average	106.5	119.17		-	

Table 3. The 5 quality traits of QTL mapping results for RILs populations in 2018 and 2019.

Trait	Year	QTL	Chr.	Marker interval	LOD	PVE (%)	Add
CP	2018	-	-	-	-	-	-
	2019	qCP4	4	15708304-61882402	5.04	16.93	0.64
NDF	2018	qNDF4	4	53612901-66114153	3.36	10.44	-14.54
		qNDF5	5	2878881-10759375	6.13	40.05	-5.94
		qNDF7	7	55751099-63896622	2.79	8.06	9.20
	2019	qNDF1	1	4353375-35252444	2.53	45.19	1.83
		qNDF4	4	53612901-66114153	3.02	7.18	-3.12
		qNDF8	8	7413025-47787281	3.45	58.74	1.86
ADF	2018	qADF5.1	5	2878881-10759375	4.84	45.26	45.26
	2019	qADF1	1	4353375-35252444	2.94	47.70	1.93
		qADF5.2	5	2878881-10759375	3.42	16.04	0.65
		qADF8	8	7413025-47787281	3.66	51.71	1.98
ADL	2018	qADL5	5	2878881-10759375	3.17	30.67	30.67
	2019	qADL1	1	4353375-35252444	2.50	25.83	0.34
HC	2018	qHC2	2	770354-72983978	3.25	6.28	2.09
		qHC3.1	3	5620530-7397531	6.61	13.64	1.58
		qHC4.1	4	54085479-55216137	3.99	7.91	2.62
		qHC4.2	4	53612901-66114153	7.08	15.34	-6.47
		qHC4.3	4	2013698-7315013	6.58	14.65	-1.43
		qHC5	5	10759375-68875912	3.69	8.85	-1.24
	2019	qHC3.2	3	5620530-7397531	2.95	10.00	0.34
		qHC2	2	57202712- 63845006	2.63	10.82	-1.36
		qHC4.2	4	53612901-66114153	2.84	9.90	-1.83

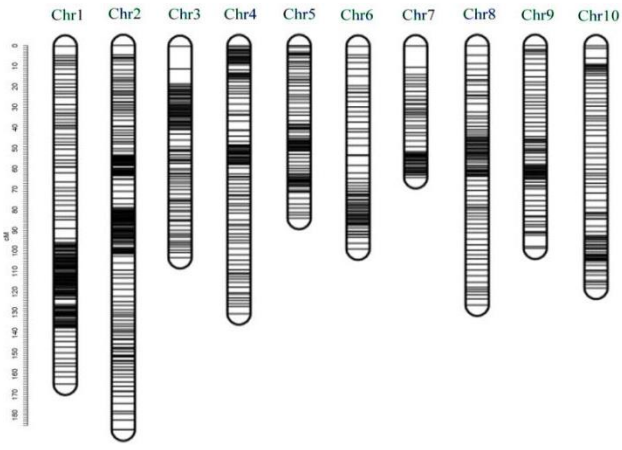

Fig. 2. The genetic density map for the RIL population.

Acid detergent fiber (ADF): Three QTLs were detected and the gene locations were on chromosomes 1, 5 and 8. The variation of phenotypic was illustrated by every QTL arranged from 16.04 to 51.71%. The maximum LOD score was 3.42, and the minimum LOD score was 2.94. Among these QTLs, qADF8 displayed the highest PVE value (51.71%). The effect of increased ADF was attributed to the three QTLs of Sa. qADL5 was detected in both years.

Acid detergent lignin (ADL): Two QTLs were detected on chromosome 1 and 5. The LOD scores was 2.50 and 3.17. Correspondly, the PVE values was 25.83% and 30.67%. The two QTLs effect of increased ADL was attributed to Sa. There was not a same QTL detected in both years of 2018 and 2019, implying stronger environment effect on ADL.

Hemicellulose (HC): There were eight QTLs were detected for HC. These QTLs were located on chromosome 1, 2, 3, 4 and 5. The mimimum LOD score was 2.63, and the maximum LOD score was 7.08. qHC4.3 showed the highest PVE (15.34%) among those QTLs. The effect of increased the content of HC was attributed to Sa at qHC2, qHC3.1, s5qHC3.2 and qHC4.1, but to the BTx623A allele at qHC2, qHC4.2, qHC4.3 and qHC5. The variation of phenotype explained by these QTLs arranged from 6.28% to 15.34%. qHC4.2 was detected in both 2018 and 2019.

The overlapping QTLs: We detected the following overlapping QTLs in this study (in pairs or triplicate): qNDF4/qHC4.2, qADF8/qNDF8, qNDF1/qADF1/qADL1, qNDF5/qADF5/qADL5 (Table 3). In these overlapping QTLs also showed high PVE (more than 20%) except qNDF4/qHC4.2. It means these QTLs can be candidate locus for forage quality breeding. All overlapping QTLs were for traits with significant correlation coefficients in both years (Fig. 1).

Discussion

Germplasm characterization is considered a prerequisite for the breeding activities as it serves as source of genetic variations (Duan *et al*., 2021; shehri *et al*., 2021; Nadeem *et al*., 2020). RAD-seq (Restriction site Associated DNA Sequencing) is a technique of reduced-representation genome sequencing. It is simple to use at low cost with high throughput (Andolfatto *et al*., 2011; Kim *et al*., 2016). In recent years, RAD-seq has been popularly applied for genetic mapping. In sorghum, the RIL population between BTx623 and NOG was used with RAD-seq to identify five QTLs for days to heading, three for plant height and total shoot fresh weight and two for Brix (Sakamoto *et al*., 2016). Zhu *et al*., also generated a highly saturated genetic map in two elite grape cultivars (Zhu *et al*., 2018). Wang *et al*., constructed a soybean genetic map and found 13 major QTLs and five QTL hotspots (Wang *et al*., 2020). In the research, we also constructed a high density genetic map with 1065 markers and mapped 19 QTLs for five quality forage traits. All these studies indicate that RAD-seq is an efficient tool to generate high-density genetics maps in crop plants.

It was co-localized for QTLs of NDF or ADF content with them of CL or HC content. The reason was NDF consisted of CL, HC and lignin, whereas ADF was consisted of CL and lignin. In sorghum, some researchers identified colocalized QTLs in relation to the content of NDF, ADF, CL and HC used QTL mapping (Murray *et al*., 2008; Shiringani & Friedt, 2011). 4 co-localized QTLs were identified: 2 QTLs colocalization of three traits and 2 QTLs colocalization of two traits. In addition, we also identified that it was significantly correlated in NDF, ADL and HC, and it was significantly correlated in ADF, ADL and HC. So, the QTLs were colocalizition which was rational.

Comparing to previous researches (Mace *et al*., 2019) in QTLs associated forage quality traits, we found that 5 QTLs were mapped the same position in their study. The qCP4 was located on the same location by Rhodes *et al*., (2017) and Murray *et al*., (2008) in sorghum as well. Two QTLs for NDF, qNDF5 and qNDF8, were located on the same region of chromosome 5 and chromosome 8 by Li *et al*., (2018). Our research also identified that the two QTLs showed high PVE. Similarly, qHC2 and qHC3.1 were mapped on chromosome 2 and chromosome 3, which was in agreement with the report of Shiringani and Friedt (Shiringani & Friedt, 2011). All these results suggested that RAD-seq can be an effective tool in mapping major QTLs in sorghum and sudangrass. It means that these QTLs controlling forage quality traits had strong effects. So, it can be detected by different researches.

Conclusions

In this study, a high density genetic map of the RIL population was constructed from sorghum Tx623A - sudangrass Sa hybrid using RAD-seq. We charactered the phenotype of five forage quality traits which included CP, NDF, ADF, ADL and HC by an Antaris™ II FT-NIR Analyzer. We also mapped 19 QTLs controlling these traits in a RIL population whose parents were Tx623A and Sa. The research results will supply very meaningful information to make better the forage quality breeding in sorghum-sudangrass hybrids.

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