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QTL mapping of forage yield and forage yield component traits in *Sorghum bicolor* x *S. sudanense*

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ABSTRACT. The sorghum-sudangrass hybrid (*Sorghum bicolor* x *S. sudanense*) is an important forage crop. However, little is known about the genetic mechanisms related to forage yield and the 4 forage yield component traits in this forage crop. In this study, a linkage map was constructed with 124 assigned SSR markers using an F_2 mapping population derived from the crossing of sorghum Tx623A and sudangrass Sa. Nine quantitative trait loci (QTLs) were detected for forage yield and the 4 forage yield component traits using inclusive composite interval mapping. Five fresh weight QTLs were identified and contributed >50% of the total phenotypic variance. Of these QTLs, all showed additive and dominant effects, but most exhibited mainly dominant effects. These results will provide useful information for improvements in sorghum-sudangrass hybrid breeding.

Key words: Sorghum-sudangrass hybrid; Simple sequence repeat; Forage yield traits; Quantitative trait locus mapping

INTRODUCTION

Sorghum (*Sorghum bicolor*) is ranked as the fifth most important grain crop worldwide; it is a major food staple and fodder resource (Xin et al., 2008). It also plays a huge role in the world market as a means of livelihood for millions of subsistence farmers (Duodu et al., 2003). Sudan grass (*S. sudanense*) and sorghum are diploids (i.e., 2n = 20) and distant species of the genus *Sorghum*. Sorghum and sudangrass are open pollinated and produce fertile offspring when crossed. The hybrid of sorghum and sudangrass (i.e., sorghum-sudangrass hybrid) is an annual forage crop with high nutritional quality, drought tolerance, lodging resistance, and high yield when compared to grain sorghum. The sorghum and sudangrass hybrid has been cultivated across 6800 hectares of land in China, to date (Yi et al., 1999).

The identification of quantitative trait loci (QTL) affecting agronomically important traits was conducted to understand their underlying genetic mechanisms and the genetic basis of their complex interactions (Srinivas et al., 2009). With the rapid development of molecular markers and linkage maps, it is possible to identify QTLs that control agronomic traits in sorghum. In recent years, a number of sorghum QTLs related to flowering time (Lin et al., 1995; Shiringani et al., 2010; El Mannai et al., 2012), drought resistance (Crasta et al., 1999; Xu et al., 2000), disease resistance (Nair et al., 2005; Wu et al., 2007; Wu and Huang, 2008), crop yield (El Mannai et al., 2012; Rajkumar et al., 2013), protein digestibility (Winn et al., 2009), sugar production (Jordan et al., 2004), plant height, and stem diameter (Lin et al., 1995; Jordan et al., 2004; Jang et al., 2006; Shiringani et al., 2010; Zou et al., 2012) were identified. To date, most of the research has focused on these traits in relation to resistance, crop yield, and plant height. However, there are limited studies investigating sorghum forage traits, especially with regard to the sorghum and sudangrass hybrid. To our knowledge, there has only been one report on the QTL mapping of agronomic traits in the *Sorghum bicolor* x *S. sudanense* hybrid. Lu et al. (2011) identified 48 QTLs of 10 agronomic traits in an F_2 population of a sorghum 314A and sudangrass 2002GZ-1 hybrid using amplified fragment length polymorphism (AFLP) and randomly amplified polymorphism DNA (RAPD) markers. Compared to AFLP and RAPD markers, simple sequence repeat (SSR) markers have more privileges for molecular assisted breeding (Yang et al., 1996).

In the present study, we constructed the first SSR genetic map of a sorghum-sudangrass hybrid. We also identified QTLs for forage yield and 4 forage yield component traits using an F_2 population of the sorghum-sudangrass hybrid. These QTLs and the flanked markers will be useful tools for improving sorghum-sudangrass hybrid breeding.

MATERIAL AND METHODS

Material

Plants from the mapping population of the F_2 population were descendants of a cross between sorghum Tx623A (i.e., female parent) and sudangrass Sa (i.e., male parent). The F_1 hybrid plants were bagged for self-pollination, and the harvested F_2 seeds were cultivated in the field in Fengyang County (China). In the summer of 2013, all generations of the mapping population (i.e., P_1, P_2, and F_2) were cultivated in Fengyang County.

Forage yield trait analysis

Ten plants were measured for P_1 and P_2; 184 plants from the F_2 population were measured. The mean, SE, range, and correlation between traits were calculated using the Excel software. Five forage yield traits were measured, including stem diameter (SD; i.e., maximum diameter of the main stem), fresh weight (FW), blade number (BN), tiller number (TN; i.e., number of tillers per plant), and plant height (PH) (i.e., length from the base to the tip of the spike).

Marker genotyping

DNAs from the parent and F_2 populations were extracted using the sodium dodecyl sulfate method (Zhen et al., 2007). A total of 924 SSR markers evenly distributed on 10 sorghum chromosomes were selected to survey polymorphisms in the parents. A 10-μL polymerase chain reaction (PCR) mixture was utilized and composed of 2.0 μL DNA (20 ng/μL), 1.0 μL 10X buffer (20 mM Mg^{2+}), 1.5 μL primers (2 pmol/μL), 1.0 μL dNTPs (1 mM), 0.5 μL rTaq (2 U/μL), and 4.0 μL ddH_2O. Samples were pre-denatured at 94°C for 5 min; followed by 36 cycles of 30 s at 94°C , 30 s at 55°C, and 80 s at 72°C; and a final extension at 72°C for 7 min. PCR products were detected using silver staining on 8% nondenaturing polyacrylamide gel.

Linkage map construction and QTL mapping

The construction of a linkage map was employed by JoinMap 4.0. Linkage groups were initially determined by the results of pair-wise comparisons at a minimum likelihood of odds (LOD) value of 2-10. The best order was determined by comparing the goodness of fit of the resulting map for each tested order using a threshold of 1.0 and 2.0 for the linkage groups and loci, respectively. Marker sites significantly associated with traits were identified. The genetic map distance in centiMorgans (cM) was calculated using the Kosambi mapping function.

QTL IciMapping 4.0 was used to detect forage traits related to QTLs. A threshold of LOD > 2.5 was used to identify the presence of putative QTLs. Inclusive composite interval mapping (ICIM) was performed to claim the putative QTLs (Wang et al., 2014).

RESULTS

Molecular linkage map of the sorghum-sudangrass hybrid

A total of 924 primers were screened between sorghum Tx623A and sudan grass Sa. We identified 180 primers that were polymorphic between the parents, and the percentage of polymorphism was 19.48%. Ultimately, the linkage map of the sorghum-sudangrass hybrid was constructed by using 124 markers and covered 1196.2 cM of the genome (Figure 1). The average interval among the markers was 10.4 cM (Figure 1). The distribution of SSR loci on the linkage map was non-random. The number of markers on individual linkage groups varied from 9(chr8) to 21(chr1).

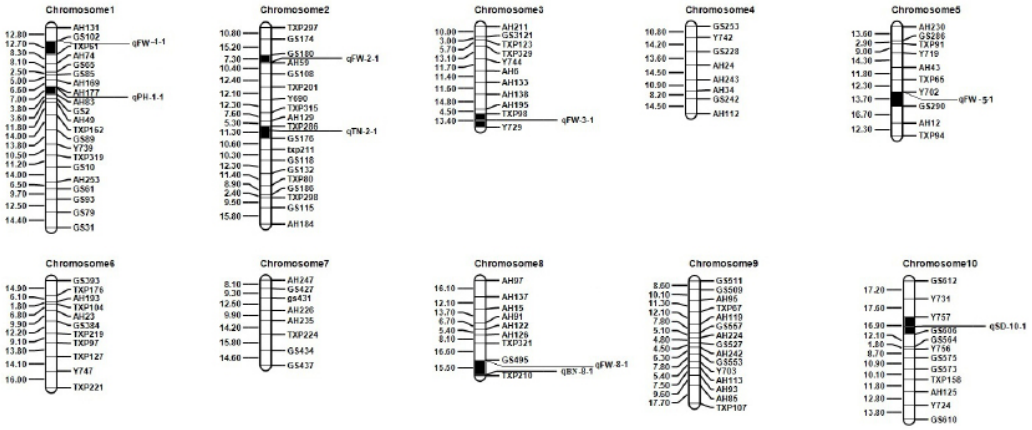

Figure 1. Position of QTLs associated with forage yield traits and 4 forage yield component traits in the F_2 population. FW = Fresh weight per plant; PH = plant height; TN = tiller number; SD = stem diameter; BN = blade number.

Trait analysis

The 2 parents and F_2 population were all measured for their phenotypic scores. The parental lines showed statistically significant differences for 5 traits. The value of skewness and kurtosis were small for the 5 traits in the F_2 population. Thus, the 5 traits showed a normal distribution in the F_2 population. Therefore, we can further map the location of the 5 traits in the population (Table 1).

Table 1. Statistical analysis of forage yield and 4 forage yield component traits in the population.

Trait name	F_2 population			Parents	
	Mean	Skewness	Kurtosis	Tx623A	Sa
Plant height (m)	2.41 ± 0.56	-0.37	0.16	1.21 ± 0.26	2.79 ± 0.31
Blade number	11.10 ± 1.43	-0.30	0.61	12.40 ± 1.52	9.00 ± 1.00
Fresh weight (kg)	1.36 ± 0.29	-0.16	-0.50	0.52 ± 0.13	1.23 ± 0.21
Stem diameter (mm)	5.00 ± 2.50	0.58	0.14	20.6 ± 0.31	11.3 ± 0.19
Tiller number	2.63 ± 1.28	0.75	0.30	1.50 ± 0.31	3.20 ± 1.30

To understand the relationship between the 5 traits, correlation analyses were performed. The results showed that plant height was significantly and positively correlated with fresh weight, stem diameter, and tiller number. Blade number was positively correlated with fresh weight and stem diameter. Fresh weight and stem diameter had a significantly positive correlation. The results showed that the other 3 traits had a significantly positive correlation with fresh weight, with the exception of tiller number.

QTL analysis

Using the 5 traits and a genetic map, 9 QTLs were identified in the F_2 population. These QTLs were distributed on chromosomes 1, 2, 3, 5, 8, and 10 (Table 2). The phenotypic variations of these QTLs were 7.46 to 29.49%.

Table 2. Correlation coefficients between five traits in the population.

	Plant height	Blade number	Fresh weight	Stem diameter	Tiller number
Plant height	1				
Blade number	0.0625	1			
Fresh weight	0.2430**	0.2828**	1		
Stem diameter	0.3893**	0.2342**	0.3001**	1	
Tiller number	0.3893**	0.0068	0.1070	0.5472**	1

**Denote significant 0.01 probability.

Plant height and blade number

One QTL on chromosome 1, at about AH177-AH83, was found to be relevant to plant height. It exhibited additive and dominant effects, but mainly showed a dominant effect. The phenotypic variation of the QTL was 7.57% (Table 3). Another QTL was related to blade number. It was localized on chromosome 8 between the marker interval GS495 and TXP210. This locus exhibited additive and dominant effects. The phenotypic variation for blade number was 7.66%; the additive was -0.12 and the dominant was 0.78.

Table 3. Putative QTLs of forage yield and 4 forage yield component traits in the population.

Trait name	Chromosome	QTL name	Marker interval	LOD	R^2 (%)	Additive	Dominant
Plant height	1	qPH-1	AH177-AH83	3.14	7.57	-0.07	-0.32
Blade number	8	qBN-8	GS495-TXP210	3.35	7.66	-0.12	0.78
	1	qFW-1	GS102-TXP61	2.79	7.46	0.03	0.16
	2	qFW-2	GS180-AH59	3.72	8.36	0.01	-0.18
Fresh weight	3	qFW-3	TXP98-Y729	4.09	14.66	-0.15	-0.11
	5	qFW-5	Y702-GS290	2.63	8.86	-0.08	-0.11
	8	qFW-8	GS495-TXP210	3.86	14.28	-0.05	0.21
Stem diameter	10	qSD-10	Y757-GS606	3.73	29.49	-0.003	0.28
Tiller number	2	qTN-2	TXP286-GS176	2.66	13.23	0.21	-0.96

Donor for the positive additive effect is from Tx623A, and donor for the negative additive effect is from Sa.

Stem diameter and tiller number

Only one QTL on chromosome 10, at about Y757-GS606, was found to be relevant to stem diameter. It exhibited additive and dominant effects. The phenotypic variation for the stem diameter was 29.49%. Thus, it can be concluded that these QTLs are major genetic loci controlling plant height in this mapping population. A QTL related to till number was detected, which was located on chromosome 2 at the interval between markers TXP286 and GS176. The phenotypic variation for tiller number was 13.23%. It showed additive and dominant effects, but the dominant effect was the main effect.

Fresh weight

Fresh weight is a direct measure of forage production. Five QTLs on chromosomes 1, 2, 3, 5, and 8, at about GS102-TXP61, GS180-AH59, Y729-TXP98, Y702-GS290, and GS495-TXP210 were detected to be relevant to fresh weight (Figure 1; Table 3). In these QTLs, the smallest and largest phenotypic variations for these QTLs were 7.46 and 14.66%, respectively. All of these QTLs for fresh weight explained 53.62% of the total phonotypic

variation. Thus, it can be concluded that these QTLs are major genetic loci controlling fresh weight in this mapping population. These QTLs showed additive and dominant effects, but the dominant effect was the main effect, with the exception of qFW-3. Thus, the dominant effect played a major role in the fresh weight traits.

In the current research, we also identified one QTL that was relevant to 2 traits. For example, the 2 markers for fresh weight and blade number were located on the chromosome 8 interval between GS495and TXP210. In addition, the 2 QTLs had primarily positive and dominant effects.

DISCUSSION

The hybrid of the sorghum and sudangrass is an important forage crop. It has been widely cultivated in China and America (Zhan et al., 2008). In comparison to other crops (e.g., rice and maize), reports on QTL mapping for forage traits in this hybrid are still relatively lacking. Lu et al. (2011) first identified 3 QTLs pertaining to fresh weight using an $F_{2:3}$ population of the sorghum-sudangrass hybrid. These QTLs for fresh weight were detected on chromosomes 1, 3, and 6. In this study, we identified 5 QTLs pertaining to fresh weight. In these QTLs, qFW-1 and qFW-3 were mapped on the same region in the current and Lu et al. (2011) research. Thus, our research is consistent with the report by Lu et al. (2011). However, in the current research, we also identified 3 new QTLs for fresh weight. In these QTLs, qFW-3 contributed 14.66% of the phenotypic variation and showed an additive effect. It may be a main-effect QTL for fresh weight. Therefore, further fine mapping of this QTL will be helpful for understanding the genetic basis for forage yield.

Pleiotropy (i.e., when one locus affects multiple phenotypic characteristics) has been an active topic of discussion in genetics. We co-located a fresh weight QTL and blade number QTL in the same region on chromosome 8. The co-location of QTLs has been reported in wheat and sorghum, especially for related traits (Mace et al., 2012; Lillemo et al., 2013). We also showed that fresh weight and blade number had a significant correlation. Blade number should be a component of fresh weight. Thus, we can easily understand why a QTL for the 2 traits were located within the same region. However, the molecular mechanism of co-location requires additional research.

Heterosis is a widely documented phenomenon in diploid organisms that undergo sexual reproduction. The genetic basis of heterosis has been debated for more than 100 years, and it is still not resolved. The dominance and overdominance hypotheses are the primary explanations for this phenomenon (Xiao et al., 1995; Meyer et al., 2010). Wancao No.3 was the F_1 hybrid of the parents in previous research. Wancao No.3 showed great heterosis especially in forage yield (Zhan et al., 2010). However, little is known on the genetic mechanisms for the variety. In this research, we identified 9 QTLs pertaining to forage yield and 4 forage component traits. In these QTLs, most of them mainly showed a dominant effect, except qFW-3. Therefore, dominance is the major genetic basis of heterosis in the sorghum-sudangrass hybrid. The same mechanisms have also been identified in rice and maize (Garcia et al., 2008). This result suggests that in the process of parent selection for breeding, we should consider the complementation of parents for forage yield traits in the sorghum-sudangrass hybrid.

Mapping of agronomic traits provides the basis for molecular-assisted breeding. In the current research, we mapped 9 QTLs for 5 traits using SSR markers. Compared to previous research using RAPD and AFLP markers, SSR markers are easier to use in molecular-assisted

breeding (Lu et al., 2011; Xu, 2011). Therefore, the flanked markers for these QTLs would be useful for molecular breeding in the sorghum-sudangrass hybrid.

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