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Fodder quality comparison in two sorghum populations under drought

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Abstract

Digestibility and lignin content are important in determining feed quality and plant fitness, with higher lignin content reducing digestibility and vice versa. Reports suggest that a variation of 5% in fodder digestibility between poor and premium fodder result in a 20% price variation. Sorghum is gaining importance as a food, feed, and fuel crop; it has similar feed quality to maize, which demands high nutrient and water availability. Additionally, drought in the semi-arid tropics is also affecting feed quality, and sorghum is known to encounter drought mainly in the post rainy season. Therefore, two sorghum populations, a recombinant inbred line (RIL) population (n=320) and reference set (n=130) were evaluated under drought for fodder qualities. In this study, irrigation was withheld at the booting stage for the stress plots, whereas the control plots were fully irrigated, all other crop management practices were performed equally. The dry weight (DW) was recorded at maturity, and the fodder was subjected to near-infrared spectroscopy (NIRS) scanning to record: nitrogen content on a dry matter basis (NDM); neutral and acid detergent fibre (NDF and ADF); acid detergent lignin (ADL); metabolizable energy (ME) and; *in vitro* organic matter digestibility (IVOMD). Significant variation was recorded across treatments and for all traits in the RIL population. Significant genotypic variation and genotype by treatment variation were recorded for the reference set in 2016 only. Pearson's correlation was pooled across years and treatments for both populations. DW and IVOMD showed negative correlations with NDF and ADL, while positive correlations were observed between DW, ME, and IVOMD in the RIL population. However, in the reference set, there was no strong positive or negative correlation between DW, ME, and IVOMD. Genotyping by sequencing (GBS) analysis was used to perform quantitative trait loci (QTL) estimation for the RIL population, while a genome-wide association study (GWAS) was performed for the reference set. A total of 98 and 47 associated genes were extracted from Phytozome v12.1.6 for the RIL and reference populations, respectively. Several genes belong to pathways that may help explain a causal functionality with the associated traits. These results will help select these traits in plant breeding programs and help achieve greater genetic gains at a faster pace.

Key word: Sorghum, drought, fodder, digestibility

Background

Sorghum is a failsafe crop in semi-arid and tropical regions as it has innate drought tolerance coupled with high water use efficiency and photosynthetic efficiency (Sunoj et al., 2020). The grain is used as food and feed, and the crop residue is used as feed for livestock. Fodder digestibility and lignin content determine the feed quality and plant

fitness, respectively. However these traits show a high trade-off. A 5% variation in fodder digestibility is reported to result in a 20% price variation for sorghum fodder (Fig. 1). Thus, this trait has a high economic impact and affects the cost and productivity of livestock (Blummel et al., 2013).

Methods

Two sorghum populations (RIL and Reference set) were evaluated under terminal drought stress by withholding irrigation at the boot leaf stage. Fodder dry weight (DW) was recorded at maturity and the fodder was subjected to near-infrared spectroscopy (NIRS) to record nitrogen content on a dry matter basis (NDM); neutral and acid detergent fibre (NDF and ADF); acid detergent lignin (ADL); metabolizable energy (ME) and; *in vitro* organic matter digestibility (IVOMD). ICIM software for QTL mapping and rMVP (R package) for GWAS were used to identify genomic regions for the traits under study. Genes were blasted against the reference genome, and *Zea mays* in Phytozome and plotted in Circos (Krzywinski et al., 2009).

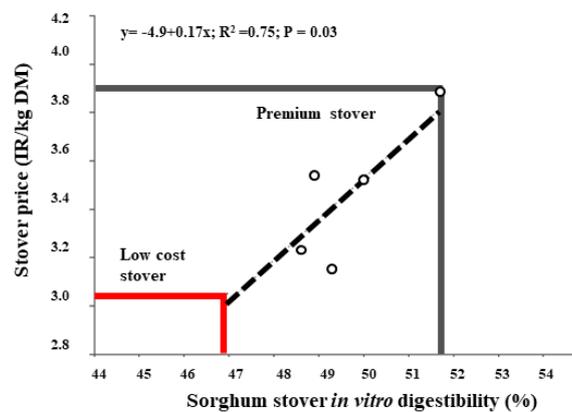


Fig 1: Relation between stover price and *in vitro* digestibility in sorghum fodder

Results

Significant genotypic variation was observed for both years; genotype by treatment significance was observed in the Reference set alone in year 2016. The second-year showed no significant variation across genotypes or treatments. The control treatment across years had no significant variation for fodder quality in the current study, but stress in the RIL population had a significant effect on all of the traits under study (Table 1).

Table 1: ANOVA for fodder quality traits across years and treatments for the RIL population and Reference set

Year	Across years				Across Treatments	
	2016		2017		Control	Stress
Traits	σ^2	σ_{gxt}^2	σ^2	σ_{gxt}^2	σ^2	σ^2
RIL population						
DW	13035**	5471**	3395**	2057**	0.3*	2202*
NDM	0.0007*	0.003934	0	0.001951	0.01	0.01*
NDF	0.784	0.979	0.03	0.388	-0.01	0.69**
ADF	0.309	1.074	0.06	0.125	-0.07	0.49**
ADL	0.007*	0.02635	0.0018	0.0049	0.01	0.01*
ME	0.007*	0.02703	0	0.00329	-0.01	0.02**
IVOMD	0.253*	0.965	0.159	0.148*	-0.11	0.38**
Reference set						
DW	12115	12231	700.81	2640.15*	23.86	451
NDM	0.000469	0.001**	0.00048	0.004**	-0.00056	-0.00014
NDF	1.56*	2.40**	-0.83	0.64	-0.03	-1.35
ADF	1.502*	1.32*	-0.35	0.06	-0.39	-0.62
ADL	0.038**	0.019*	0.0003	-0.0019	0.0088	-0.0083
ME	0.045**	0.038**	0.0154	-0.0234	-0.0138	-0.0146
IVOMD	1.57**	1.48**	0.15	0.08	-0.482	-0.555

σ^2 = genotypic variance; σ_{gxt}^2 genotypic x treatment variance= * Significant at $P < 0.05$, ** Significant at $P < 0.01$

Pearson's correlation across years and treatments for DW and IVOMD showed negative correlations with NDF and ADL (Table 2). Six genes were mapped for IVOMD, on chromosomes 1, 2, 5, 7, 8, and 9, Sobic.001G530300 and Sobic.008G054500 mapped under drought stress, and three genes each were identified under drought stress on chromosome 1 and 2 for IVOMD. Sobic.001G354800, which is associated with seed-dry grain maturity was mapped against ADL, IVOMD, and ME under drought stress (Table 3).

Table 2: Correlation between fodder quality traits for both populations under study.

Pop	Trait	NDF	ADL	ME	IVOMD
REF	DW	0.00	0.04	0.07	0.07
	NDF		0.60**	-0.71**	-0.78**
	ADL			-0.75**	-0.75**
	ME				0.97**
RIL	DW	-0.07	-0.14*	0.28**	0.19**
	NDF		0.60**	-0.70**	-0.75**
	ADL			-0.79**	-0.79**
	ME				0.97**

Table 3: Genes identified for DW and IVOMD using significant positions from QTL mapping and GWAS

Pop	Trait	Chr	Gene ID	Pop	Trait	Chr	Gene ID
REF	IVOMD_C_17.FarmCPU	Chr01	Sobic.001G180700	REF	DW_S_17.FarmCPU	Chr01	Sobic.001G279000
REF	IVOMD_S.FarmCPU	Chr01	Sobic.001G530300	REF	DW_S_17.FarmCPU	Chr02	Sobic.002G249600
REF	IVOMD_C_17.FarmCPU	Chr02	Sobic.002G231800	REF	DW_S_17.FarmCPU	Chr04	Sobic.004G046900
REF	IVOMD_C_17.FarmCPU	Chr07	Sobic.007G023400	REF	DW_S_17.FarmCPU	Chr04	Sobic.004G046900
REF	IVOMD_S_16.FarmCPU	Chr08	Sobic.008G054500	REF	DW_S_17.FarmCPU	Chr05	Sobic.005G196500
REF	IVOMD_S.FarmCPU	Chr09	Sobic.009G217500	REF	DW_S_17.FarmCPU	Chr05	Sobic.005G196500
RIL	q_IVOMD_16_S_1_1	Chr01	Sobic.001G354800	REF	DW_S_17.FarmCPU	Chr08	Sobic.008G116300
RIL	q_IVOMD_17_S_1_2	Chr01	Sobic.001G463900	REF	DW_C_17.FarmCPU	Chr09	Sobic.009G206700
RIL	q_IVOMD_17_S_1_3	Chr01	Sobic.001G463900	RIL	q_DW_17_S_2_1	Chr02	Sobic.002G073700
RIL	q_IVOMD_17_S_1_1	Chr01	Sobic.001G472301	RIL	q_DW_17_C_3_1	Chr03	Sobic.003G280500
RIL	q_IVOMD_S_2_1	Chr02	Sobic.002G222500	RIL	q_DW_17_S_3_1	Chr03	Sobic.003G280500
RIL	q_IVOMD_16_S_2_1	Chr02	Sobic.002G335900	RIL	q_DW_C_3_1	Chr03	Sobic.003G291700
RIL	q_IVOMD_S_2_2	Chr02	Sobic.002G350600	RIL	q_DW_C_3_2	Chr03	Sobic.003G407800
RIL	q_IVOMD_16_S_4_1	Chr04	Sobic.004G180200	RIL	q_DW_17_S_5_1	Chr05	Sobic.005G010900
RIL	q_IVOMD_16_C_5_1	Chr05	Sobic.005G153033	RIL	q_DW_16_C_7_1	Chr07	Sobic.007G099700
RIL	q_IVOMD_C_5_1	Chr05	Sobic.005G172400	RIL	q_DW_S_7_1	Chr07	Sobic.007G099700
RIL	q_IVOMD_17_C_6_1	Chr06	Sobic.006G014700	RIL	q_DW_17_C_7_1	Chr07	Sobic.007G103600
RIL	q_IVOMD_16_C_7_1	Chr07	Sobic.007G098800	RIL	q_DW_C_7_2	Chr07	Sobic.007G136300
RIL	q_IVOMD_S_8_1	Chr08	Sobic.008G026400	RIL	q_DW_17_S_7_1	Chr07	Sobic.007G145600
RIL	q_IVOMD_16_C_10_1	Chr10	Sobic.010G107100	RIL	q_DW_16_C_7_2	Chr07	Sobic.007G146200
RIL	q_IVOMD_C_10_1	Chr10	Sobic.010G139600	RIL	q_DW_16_C_7_3	Chr07	Sobic.007G153900
RIL	q_IVOMD_16_S_10_1	Chr10	Sobic.010G229000	RIL	q_DW_17_C_10_1	Chr10	Sobic.010G229000

The gene Sobic.007G023400, mapped to IVOMD under control conditions in the Reference set, is linked to the super pathway of cytosolic glycolysis (plants), pyruvate dehydrogenase and TCA cycle, glyoxylate cycle and fatty acid degradation and aerobic respiration I, II and III. ME for the Reference set under stress was associated with Sobic.003G282600, a gene linked to homogalacturonan biosynthesis which contributes to plant growth and development and cell wall structure. Dry weight in the RIL population was associated with Sobic.007G145600 and Sobic.007G146200, which are involved in multiple pathways associated with cell wall-bound phenolic acids that play a major role in plant defense against pathogens, mapped on chromosome 7. ADL under stress in the RIL population was linked to Sobic.009G148200 on chromosome 9, associated with the pyrimidine and purine deoxyribonucleoside salvage pathway which is notably triggered under stress. These results will help in trait manipulation during plant breeding practices and to achieve genetic gains at a faster pace.

In Fig. 2, genes in the genomic regions associated with DW, IVOMD, and combined are indicated, while the unmarked regions contain genes associated with DW. The genes Sobic.010G229000 and Sobic.002G335900 were associated with both key traits in the RIL population, while no such gene associations were identified in the Reference set. Dry weight in the RIL population was associated with Sobic.007G145600 and Sobic.007G146200, involved in the production of cell wall-bound phenolic acids that play a major role in defense against pathogens, all on chromosome 7. ME in the Reference set under stress was associated with Sobic.003G282600, a gene linked to homogalacturonan biosynthesis that contributes to plant growth and development and cell wall structure. The syntenic relation between sorghum and maize showed that Sobic.007G023400, a gene associated the IVOMD, shared 97.2 % similarity with the maize gene GRMZM2G134134_T02, while Sobic.009G206700, associated with DW, shared 98.6% similarity with maize gene GRMZM2G319747_T02.

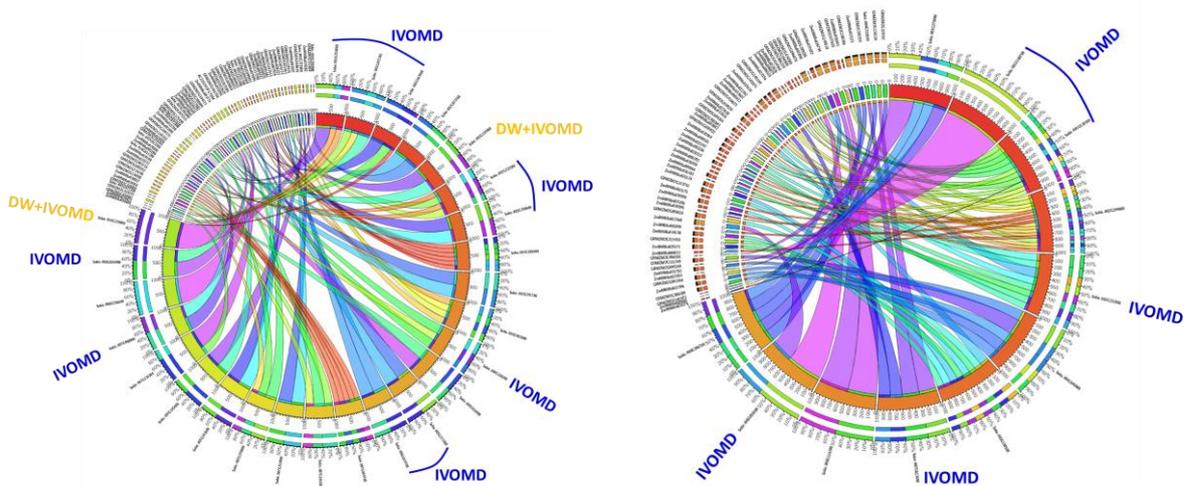


Fig 2: Synteny between sorghum RIL population (left) and Reference Set (right) with *Zea mays*

Conclusion

The concurrent improvement of fodder dry weight and IVOMD in sorghum crop improvement is possible. A gene, Sobic.001G356000, was linked to lignin and metabolizable energy traits under stress conditions; this can be key to understanding their behavior and for the use of genetic tools to reduce the negative impacts observed. While the gene Sobic.001G463900 was mapped to *in vitro* organic matter digestibility, further fine mapping for IVOMD on chromosomes 1 and 2 will improve the opportunity for trait dissection.

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