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Summary

A factor frequently identified as a key to understanding sorghum [*Sorghum bicolor* (L.) Moench] feed value to ruminant animals is rate of starch digestion. Recent research in corn (*Zea mays* L.) has established a strong ability to predict rumen starch degradation from grain physical and chemical parameters. It was therefore important to determine whether similar relationships could be established in sorghum. The objectives of this study were to determine: 1) range of variation for 12-hour *in vitro* dry matter disappearance (IVDMD), chemical, and physical grain quality parameters among 16 sorghum conversion lines; and 2) to establish the strength of the relationships of these characters. Entries were grown at Ithaca, Nebraska in 1991 and 1992 in a randomized complete block with four replications. Line effects were significant for 12-hour IVMVD, crude protein, oil, starch, individual seed weight, and hardness. The only traits significantly correlated with 12-hour IVDMD were crude protein and hardness, with $r \leq -0.32$ for testa and non-testa-containing lines. Stepwise regression similarly revealed poor predictive ability for any of the traits on 12-hour IVDMD. Unlike corn, prediction of digestibility from simply measured physical parameters was not possible in this set of 16 sorghum lines. However, the lack of strong relationships provides opportunity to select lines with unique combinations of traits for individual targeted needs or markets.

Abbreviations: SKWCS – Single Kernel Wheat Characterization System; IVDMD – *in vitro* dry matter disappearance

Introduction

Understanding the relationships between sorghum [*Sorghum bicolor* (L.) Moench] physical and chemical characteristics, with the concomitant goal of predicting livestock performance, has received significant research attention in recent years. One factor frequently identified as a key to understanding sorghum feed value to ruminant animals is rate of starch digestion. Wester et al. (1992) showed significant genetic variation in sorghum for *in vitro* rate of starch disappearance and a strong relationship between *in vitro* rate of starch disappearance and feed/gain ration (R^2 = 0.94) in the feedlot. However, despite considerable technique refinement (Richards et al., 1995) measurement of *in vitro* rate of starch disappearance in high-volume high-throughput research has remained impractical due high labor and other inputs.

Kotarski et al. (1992) demonstrated a higher *in vitro* rate of starch disappearance in a sorghum line with floury endosperm when compared to a sorghum line with vitreous endosperm. Pedersen et al. (1996) demonstrated considerable variation in % vitreous endosperm among 16 sorghum conversion lines grown in a single year with % vitreous endosperm ranging form 53 to 93%. They also verified that a new device with very high throughput potential, the single kernel wheat characterization system (SKWCS), could accurately measure sorghum grain hardness, diameter, and seed weight, and that these values were highly correlated to traditional laboratory measurements of the same traits.

Pedersen et al. (2000) describe a 12-hour *in vitro* dry matter disappearance (IVDMD) procedure and used it to establish a strong correlation of starch and dry matter digestion at 12 hours, the approximate retention time for grain concentrates in the rumen (Sniffen et al., 1992). Recent research in corn (*Zea mays* L.) has established a strong predictive ability of rumen starch degradation from grain vitreousness alone ($r^2 = 0.89$), or from a combination of apparent grain density and 1000-seed weight ($R^2 = 0.91$) in a group of 14 corns differing in endosperm texture (Philippeau et al., 1999). It is important to establish whether similar relationships can be established among sorghum digestibility, chemical, and physical factors. The objectives of this study were to determine: 1) range of variation for 12-hour IVDMD, chemical, and physical grain quality parameters among 16 sorghum conversion lines; and 2) to establish the strength of the relationships of these characters and their predictive value for 12-hour IVDMD.

Materials and methods

Subsets of the samples used in this study were previously used for validation of the SKWCS for sorghum (Pedersen et al., 1996) and for development of the 12-hour *in vitro* procedure (Pedersen et al., 2000). Although portions of the materials and methods used were therefore previously described, they are repeated herein for completeness and for the reader's convenience. Sixteen sorghum lines from five races and eight working groups were selected from the U.S. Department of Agriculture, Agriculture Research Service Genetic Resources Information Network data base (USDA, 2001) for purported diversity in grain size and hardness. Lines were visually classified for presence or absence of a highly pigmented testa layer after scraping away pericarp.

Entries were planted at the University of Nebraska Field Laboratory in Ithaca, NE in 7.6-m rows spaced 76 cm apart in a randomized complete block with four replications in each of two years. Planting dates were 21 May 1991 and 13 May 1992. Plots were fertilized with 112 kg ha^{-1} N prior to planting. For weed control, propachlor [2-chloro-*N*- (1-methylethyl)-*N*-phenylacetamide] and atrazine [6-chloro-*n*-ethyl-*N*'-(1-methylethyl)-1,3,5-triazine-2,4,diam ine] were applied at 3.36 and 1.12 kg ha^{-1}, respectively, immediately after planting. No supplemental irrigation was applied. Plots were harvested with a small-plot combine and grain was stored at 8 °C until used in this study.

Prior to collecting data on grain physical characters, subsamples from each plot were hand-cleaned each year to remove broken pieces of seed and debris, and then stored in a single sealed container for one week before measurement to allow moisture levels to equilibrate. Approximately 300 seeds from each sample were analyzed for grain hardness, diameter, and weight using the SKWCS as per Pedersen et al. (1996).

Subsamples were prepared for 12-hour IVDMD analyses by grinding to pass a 2-mm screen in a Wiley mill. *In vitro* digestion at 12 hours was determined as per Pedersen et al. (2000). Rumen fluid inoculum was obtained from a fistulated steer maintained on a grain diet. All samples from both field production years were analyzed in a single lab run to eliminate the potential for confounding of year and lab run. Total starch was determined as per Xiong et al. (1990). Crude protein (N $\times$ 6.25) was determined using a LECO Model FP428 (LECO Corporation, St. Joseph, MI). Oil was determined as using an ISCO SFX3560 (ISCO Incorporated, Lincoln, NE).

All chemical, physical and digestibility parameters were subject to analyses of variance using the GLM procedure of SAS (1990). Lines and years were considered random effects. Testa layer class (absent or present) was considered a fixed effect, and lines were nested in testa layer class. Appropriate test terms were selected for the mixed model using the RANDOM statement in GLM. LSDs were calculated for line effects to assist in data interpretation. Since the high tannin content associated with presence of pigmented testa is usually associated with reduced digestibility (Hancock, 2000), data was sorted and analyzed by testa class to establish relationships among parameters within each testa layer class. Pearson correlations were determined for the individual chemical and physical parameters with 12-hour IVDMD using the CORR procedure of SAS. Multiple linear regression equations to predict 12-hour IVDMD were built using the stepwise option in the REG procedure of SAS. Chemical and physical parameters were added until no remaining variable produced a significant ($p = 0.05$) F-statistic.

Table 1. ANOVA for digestibility, physical, and chemical grain quality parameters of 16 sorghum conversion lines

Source	df	Type III mean squares						
		12-hour IVDMD	Crude protein	Oil	Starch	Kernel diameter	Kernel hardness	Kernel weight
Total	125							
Year	1	0.51	9.04	0.84	36.40	0.701	5688	33.32
Rep(year)	6	42.23*	0.43	0.14*	11.07*	0.009	140*	9.0*
Testa	1	66.19	4.70	2.78*	138.45*	0.224	457	189.37
Line(testa)	14	29.42*	4.74*	0.57*	21.35*	1.322*	1014*	467.04*
Year*Line(testa)	15	23.48	0.69	0.05	6.14*	0.034*	176*	13.42*
Error	88	15.00	0.42	0.05	2.64	0.009	24	4.31

* Significant at $p = 0.05$.

Results

Results of Analysis of Variance are shown in Table 1. Year × line interactions were significant ($p = 0.05$) only for starch, seed diameter, seed hardness, and seed weight. Examination of the individual year data showed most lines to be ranked similarly for these parameters with small shifts in mean values in individual years. Since no year interactions were significant for the trait of primary interest, 12-hour IVDMD, and no major shifts in the relative ranking of lines for the other traits were observed, data for all traits was pooled across years to give the best estimates of 12-hour IVDMD.

Line effects were significant for all parameters. Means and LSDs are shown for each line in Table 2. Although testa effects were significant only for oil and starch content in this study, results are reported and discussed by testa class in recognition of the commercial importance of segregating sorghum grain containing testa. Non-testa sorghum lines ranged in 12-hour IVDMD from 201 to 261 g kg^{-1} with mean of 231 g kg^{-1}. This represents an extended range from that previously reported in our lab (Pedersen et al., 2000) but regretfully extends the range downward by including non-testa lines of lower 12-hour IVDMD than described in the previous report. Testa containing lines ranged in 12-hour IVDMD from 190 to 251 g kg^{-1} with a mean of 215. As expected, four of the five testa containing lines rank among the lowest of the 16 total lines examined in the study. Line SC334, however, has a highly pigmented testa yet ranks among the most digestible of the 16 lines with 12-hour IVDMD of 251 g kg^{-1}.

Crude protein, oil, and starch content fall within expectations based on previously reported values (Wall & Blessin, 1970; Waniska & Rooney, 2000). In this study, crude protein ranged from 106 to 128 g kg^{-1} with a mean of 117 g kg^{-1} for non-testa lines, and 107 to 124 g kg^{-1} with a mean of 114 for testa-containing lines. Oil ranged from 26 to 35 g kg^{-1} with a mean of 30 g kg^{-1} for non-testa lines, and 31 to 36 g kg^{-1} with a mean of 34 for testa containing lines. Starch ranged from 624 to 662 g kg^{-1} with a mean of 640 g kg^{-1} for non-testa lines, and 586 to 633 g kg^{-1} with a mean of 617 for testa containing lines.

Seed weight also falls within expectations based on previously reported values (Miller, 1974). Individual seed weights ranged from 16 to 40 mg for non-testa lines with a mean of 29 mg, and from 21 to 27 mg with a mean of 26 mg for testa containing lines. Although often discussed, little is published documenting variation in seed size (diameter) in sorghum. One reference mentions an unpublished survey of 280 commercial and experimental hybrids that ranged in seed size from 2.36 to 4.79 mm (Wills & Ali, 1982). The conversion lines examined in this study ranged in seed diameter from 1.9 to 3.3 mm with a mean of 2.5 mm for non-testa lines, and 2.1 to 3.0 mm with a mean of 2.4 mm for testa containing lines. SKWCS hardness values are slightly lower than previously reported for the same lines (Pedersen et al., 1996) because of the effect of including a second year's data in this study with average hardness values 15 SKWCS units lower. When averaged over the two years of this study, hardness of non-testa lines ranged from 54 to 105 SKWCS units with a mean of 82 SKWCS units, and testa containing lines ranged from 66 to 87 SKWCS units with a mean of 79 SKWCS units.

Table 2. Digestibility, physical, and chemical grain quality parameters of 16 sorghum conversion lines

Line	Testa layer	12-hour IVDMD (g kg^{-1})	Crude protein (g kg^{-1})	Oil (g kg^{-1})	Starch (g kg^{-1})	Kernel		
						Diameter mm	Hardness scale*	Weight mg
SC252	Absent	201	128	29	626	1.9	105	16
SC723	Absent	212	130	26	628	2.8	86	36
SC905	Absent	217	115	32	624	2.8	85	35
SC215	Absent	217	122	29	631	2.6	87	26
SC543	Absent	232	112	32	647	2.5	82	30
SC203	Absent	235	109	27	660	3.3	74	40
SC835	Absent	236	124	34	631	2.0	93	20
SC367	Absent	242	126	31	652	3.0	72	36
SC761	Absent	247	116	29	657	2.8	76	34
SC242	Absent	249	120	29	631	2.0	90	17
SC460	Absent	261	106	35	662	2.3	54	26
SC655	Present	190	124	36	586	2.4	71	25
SC949	Present	201	111	36	609	2.1	85	21
SC760	Present	216	115	33	633	2.5	83	27
SC101	Present	220	111	31	632	2.2	87	21
SC334	Present	251	107	33	627	3.0	66	37
LSD ($p = 0.05$)	–	32	5	2	14	0.1	4	2

* Hardness scale with relative values of soft wheat = 50, hard wheat = 100.

Table 3. Correlation coefficients for 12-hour IVDMD, physical, and chemical grain quality parameters of 11 non-testa (above diagonal) and 5 testa containing (below diagonal) sorghum conversion lines

	12-hour IVDMD	Crude protein	Oil	Starch	Kernel diameter	Kernel hardness	Kernel weight
12-hour IVDMD		r = –0.301 *p* = 0.005	r = 0.091 *p* = 0.402	r = 0.197 *p* = 0.067	r = 0.098 *p* = 0.380	r = –0.283 *p* = 0.010	r = 0.115 *p* = 0.305
Crude protein	r = –0.243 *p* = 0.023		r = –0.164 *p* = 0.130	r = –0.477 *p* = 0.001	r = –0.431 *p* = 0.001	r = 0.569 *p* = 0.001	r = –0.426 *p* = 0.001
Oil	r = 0.094 *p* = 0.389	r = –0.096 *p* = 0.379		r = –0.076 *p* = 0.482	r = –0.311 *p* = 0.004	r = –0.134 *p* = 0.228	r = –0.216 *p* = 0.051
Starch	r = 0.173 *p* = 0.109	r = –0.423 *p* = 0.001	r = –0.106 *p* = 0.326		r = 0.369 *p* = 0.007	r = –0.381 *p* = 0.001	r = 0.367 *p* = 0.001
Kernel diameter	r = 0.092 *p* = 0.412	r = –0.422 *p* = 0.001	r = –0.321 *p* = 0.003	r = 0.312 *p* = 0.004		r = –0.420 *p* = 0.001	r = 0.958 *p* = 0.001
Kernel hardness	r = –0.325 *p* = 0.003	r = 0.563 *p* = 0.001	r = –0.004 *p* = 0.975	r = –0.412 *p* = 0.001	r = –0.467 *p* = 0.001		r = –0.423 *p* = 0.001
Kernel weight	r = 0.131 *p* = 0.240	r = –0.419 *p* = 0.001	r = –0.218 *p* = 0.049	r = 0.322 *p* = 0.003	r = 0.954 *p* = 0.001	r = –0.454 *p* = 0.001	

Simple correlation coefficients for the various traits and their level of significance are shown for non-testa and testa containing sorghum in Table 3. Within the non-testa and testa containing subsets of samples, the only traits significantly correlated with 12-hour IVDMD were crude protein (r = –0.301 and r = –0.243, respectively) and hardness (r = –0.283 and r = –0.325, respectively). Although significant, neither trait was strongly correlated with 12-hour IVDMD. In fact, few strong correlations were found among any traits measured. Significant correlations were found between crude protein all other traits except oil. Seed size and weight were strongly correlated in both data subsets (r = 0.958 and r = 0.954, respectively), and both seed size and seed weight were showed significant correlations with and all other traits except 12-hour IVDMD.

Stepwise regression similarly revealed little predictive ability of any for the traits on 12-hour IVDMD. For the not-testa containing subset at $p = 0.05$, only hardness was included in the model [12-hour IVDMD = 29.05–0.07 (Hardness)] with $R^2 = 0.08$. For the testa containing subset at $p = 0.05$, only Oil was included in the model [12-hour IVDMD = 36.64–4.42(Oil)] with $R^2 = 0.12$.

Discussion

Unlike Philippeau et al. (1999) who reported strong relationships between starch digestion and physical characteristics in corn, we observed no strong relationships between 12-hour IVDMD and physical or chemical characteristics of sorghum grain. A key difference between the studies could lie in the reasons for, and relative amounts of variability within the sets of lines chosen for the two studies. Philippeau et al. reported a much wider range of crude protein (87–135 g kg^{-1}), starch (601–720 g kg^{-1}), and hardness (38.5–79.1% vitreousness) among their 14 experimental corn hybrids than we observed among our 16 sorghum lines. Similarly, they report a wide relative range in dry matter digestibility (397–715 g kg^{-1}), and starch digestibility (406–776 g kg^{-1}). Kernel weights for corn lines were less variable in a relative sense [(maximum-minimum)/minimum = 0.57] than for our sorghum lines [(maximum-minimum)/minimum = 1.50], but individual kernel weights and therefore the absolute range was roughly a factor of 10 higher for individual kernel weights of corn.

Although Philippeau et al. (1999) do not give information about the relative amount of genetic diversity present in their set of experimental hybrids, it is probably safe to assume that it is less than that represented by our 16 sorghum conversion lines which included five sorghum races from eight working groups; Sumac, Durra, Roxburghii, Caudatum, Caudatum-Kaura, Conspicuum, Caudatum-Kafir, and Nigricans-Bicolor entries. One must therefore ask what other factor is contributing to the wider range in variability for digestibility, and physical and chemical traits in corn than in sorghum grain. We hypothesize that the larger caryopsis of corn provides greater opportunity for expression of differences in these traits.

In a study designed to investigate the potential to select for improved protein digestibility in a random mated population of grain sorghum, Bramel-Cox et al. (1990) showed generally weak phenotypic and genetic correlations among grain yield, protein concentration, and protein digestibility, as well as a strong undesirable relationship between protein digestibility and late maturity. They suggested that breeding progress could be made using a selection index for several traits and concurrently restricting selection to families that meet minimum criteria for other traits. Beta et al. (2001) more recently found a general lack of correlation among sorghum starch properties and physical grain quality traits and suggested selection of sorghum genotypes based on specific desired end use attributes.

In this study, we too showed generally weak correlations among 12-hour IVDMD, physical, and chemical traits of sorghum grain. Based on these results, it should be possible to select individual lines possessing desirable attributes for several traits. For example, SC242 has high 12-hour IVDMD, high crude protein, does not contain a pigmented testa, and is quite hard. It should have good feed value and would combine and process without appreciable cracking and breaking of seeds.

It may also be possible to select individual lines containing a unique set of offsetting characteristics for special applications and markets. For example, SC334 contains a highly pigmented testa. However, SC334 is very soft and very digestible and may have value in new specialty applications capitalizing on the high antioxidant potential of highly pigmented testa-containing sorghum.

In conclusion within a set of 16 widely divergent sorghum conversion lines, significant variation was found for 12-hour IVDMD, chemical, and physical grain quality parameters. The relative amount of variation was less than present in corn for the same or similar traits. Unlike corn, few strong correlations among these traits were identified, and prediction of digestibility from simply measured physical parameters was not possible in this set of sorghum lines. However, the lack of strong relationships among these grain quality traits provides opportunity to select lines with unique combinations of traits for individual targeted needs or applications.

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