NOTE

Application of the Single Kernel Wheat Characterization Technology to Sorghum Grain[1]

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ABSTRACT

A single kernel wheat characterization system (SKWCS) was recently developed by the USDA, ARS Grain Marketing Research Laboratory and is currently being marketed by Perten Instruments North America, Inc. This device has been shown to accurately measure individual seed hardness, moisture, and size of wheat. The objective of this study was to determine if the SKWCS technology could be applied to the measurement of sorghum grain. Grains from 64 sorghum plots grown at Mead, NE in 1992 were characterized using a prototype SKWCS at the USDA, ARS Grain Marketing Research Laboratory. Problems encountered were primarily associated with the single kernel feeder mechanism. Occasionally, two sorghum seeds were fed to the crushing device instead of a single kernel. These double sampling events were easily detected by examination of the size data, and software limits could be set to exclude such double sampling events from the data set. If broken seeds were not removed prior to measurement of the grain, errors in hardness and size values also occurred. These errors could usually be detected by examination of the data, and eliminated by adjustment of software limits. Inspection and hand cleaning of samples is highly recommended prior to characterization. Based on our results, SKWCS technology can be successfully applied to sorghum seed.

A single kernel wheat (*Triticum aestivum* L.) characterization system (SKWCS) was recently developed by the USDA, ARS Grain Marketing Research Laboratory (Martin et al 1993) and is currently being marketed by Perten Instruments. This device has been shown to rapidly and accurately measure individual wheat seed hardness, moisture, weight, and size at a rate of approximately 110 kernels per minute. Seed hardness, weight, and size are also important parameters for assessment of sorghum [*Sorghum bicolor* (L.) Moench] grain quality (House 1985). In addition to average values for hardness, weight, and size, uniformity of these characters is important in sorghum grain quality assessment because of its impact on processing.

Application of the SKWCS to sorghum grain would provide plant breeders, grain processors, and feeders with a commercially available device to rapidly and accurately measure individual seed hardness, weight, and size of sorghum grain. The objective of this study was to determine whether SKWCS technology could be applied to the measurement of sorghum grain.

MATERIALS AND METHODS

Sixteen sorghum lines were selected from the U. S. Department of Agriculture, Agricultural Research Service Genetic Resources Information Network data base for diversity in grain size and hardness (Table I). These lines were grown at Mead, NE, in 1992 in a randomized complete block design replicated four times. All samples were hand-cleaned to remove pieces of seed and debris. All 64 samples were stored in a single sealed container for one week before measurement to allow moisture levels to equilibrate. Approximately 300 seeds from each of these 64 plots were measured for hardness, weight, and diameter using a prototype SKWCS at the USDA, ARS Grain Marketing Research Laboratory (hereafter referred to as the SKWCS). Although not measured directly by the SKWCS, density was calculated on a per sample basis by dividing average seed weight by average seed volume ($4/3\pi r^3$, assuming the seed to be spherical and r = 1/2 SKWCS diameter). Individual sample SKWCS data sets were visually examined for outliers (for example, double-sampling events) and outliers were eliminated from the data set.

For comparison with SKWCS values, hardness was assessed by quantification of seed vitreousness. The floury or opaque portion of the sorghum endosperm is usually located in the center of the grain adjacent to the embryo, with the vitreous portion being peripheral (Rooney and Miller 1982). Therefore, an adaptation of the camera lucida method of Kirleis et al (1984) was used to determine percent vitreousness. Ten representative seeds of each sample were bisected transversely with a razor blade such that the cut surface revealed the widest portion of the seed including a small segment of the germ. The seed halves were attached to cards with double-sided tape and examined with a dissecting stereo microscope (American Optical) equipped with a camera lucida (Spencer). Oblique illumination from above allowed easy differentiation between vitreous and floury endosperm. The endosperm of each seed was circumscribed, and the discrete boundary between the vitreous and floury portions was traced. Areas on the resulting drawings were integrated with a SigmaScan digitizing system (Jandel Scientific) and the ratio of vitreous to total endosperm area was calculated. Although the 16 sorghum lines used in this study were selected for their diversity in hardness, the percent vitreousness of their median transverse sections ranged only from 53.1% for the most floury line to 93.4% for the most vitreous.

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One hundred individual seeds per sample were also measured for weight with an analytical balance, and diameter with a digital caliper. Density was measured on a per sample basis with a Beckman 930 Air Comparison Pycnometer (Beckman Instruments, Inc., Fullerton, CA) using the standard American Society for Testing Materials procedure (ASTM 1994).

All calculations were made using SAS (SAS Institute 1990). Differences among lines for each trait were determined using the MEANS/DUNCAN option of GLM. Since SKWCS density values were calculated from sample means and pycnometer readings were made on a sample (rather than individual seed) basis, sample standard deviations for density values were not determined. Simple correlations of SKWCS values and traditional lab values were determined using PROC CORR. Rank correlations were determined using PROC CORR SPEARMAN. All correlations were calculated using individual sample data.

RESULTS AND DISCUSSION

Means and mean sample standard deviations for the 16 sorghum lines are shown in Tables I and II Separation of the lines into distinct groups using the Duncan mean separation procedure was at least as effective using the SKWCS as traditional laboratory measurements of grain parameters. Since SKWCS hardness and percent vitreousness are measured in different units, direct comparison of values was not attempted. Seed weights appear equivalent when measured with the SKWCS or an analytical balance. Mean seed diameters were smaller when measured with the SKWCS than when measured with the calipers. This is probably due to known errors associated with internal data acquisition and internal scaling to normalize the individual instrument to a selected reference. Although the scaling error in SKWCS diameter measurement was not deemed to be a serious problem in

TABLE I
Mean Seed Hardness, Diameter, Weight, and Density of 16 Sorghum Lines Using the Single Kernel Wheat Characterization System (SKWCS) and Traditional Laboratory Measurements[a]

	SKWCS				Traditional Laboratory Measurements			
Line	Hardness (scale)	Diameter (mm)	Weight (mg)	Density (g/cm^3)	Hardness (scale)	Diameter (mm)	Weight (mg)	Density (g/cm^3)
SC101	94cd[b]	2.06f	19.6g	4.27cd	67.2ef	3.07fg	20.6f	1.397b–d
SC203	78f	3.06a	37.0a	2.47g	62.4g	4.33ab	42.9a	1.392c–e
SC215	92cd	2.42d	25.0ef	3.36ef	68.1d–f	3.45e	24.8e	1.390c–e
SC242	108b	1.99fg	16.9h	4.10d	86.0b	2.71h	16.4gh	1.415a
SC252	116a	1.83h	15.5h	4.87b	93.4a	2.84gh	14.4h	1.421a
SC334	71g	3.01a	37.8a	2.63g	62.2g	4.00c	38.2b	1.369fg
SC367	86de	3.06a	38.0a	2.55g	67.0ef	4.38a	43.1a	1.396b–e
SC460	67g	2.22e	27.2d	4.79b	64.9fg	4.17a–c	30.1d	1.373fg
SC543	79f	2.40d	30.8c	4.25cd	73.1c	3.94cd	33.7c	1.393c–e
SC655	74fg	2.25e	24.1f	4.03d	53.1h	3.67de	25.4e	1.363g
SC723	88d	2.62cd	33.6b	3.59e	70.9c–e	4.05c	34.2c	1.382d–f
SC760	89d	2.43d	26.2de	3.51ef	61.6g	3.71de	26.0e	1.383d–f
SC761	80ef	2.75b	33.9b	3.14f	67.9d–f	4.08bc	35.3c	1.381ef
SC835	97c	1.90gh	18.9g	5.28a	71.7cd	3.19f	18.5fg	1.399bc
SC905	91cd	2.70bc	34.2b	3.35ef	72.4c	3.98c	33.9c	1.407ab
SC949	93cd	2.04f	20.4g	4.58bc	69.6c–e	3.05fg	19.3f	1.410a

[a] Hardness measured as % vitreous endosperm from camera-lucida drawings of longitudinally dissected seeds. Diameter measured longitudinally with a caliper. Weight measured with an analytical balance. Density measured with a pycnometer. SKWCS hardness relative scale based on soft wheat = 50, hard wheat = 100.

[b] Means in a column followed by the same letter are not significantly different at $P = 0.05$ using Duncan's mean separation test.

TABLE II
Mean Sample Standard Deviation for Seed Hardness, Diameter, Weight, and Density of 16 Sorghum Lines Using the Single Kernel Wheat Characterization System (SKWCS) and Traditional Laboratory Measurements[a]

	SKWCS				Traditional Laboratory Measurements			
Line	Hardness (scale)	Diameter (mm)	Weight (mg)	Density (g/cm^3)	Hardness (scale)	Diameter (mm)	Weight (mg)	Density (g/cm^3)
SC101	20a[b]	0.29e–g	4.1g–i	. . .	6.7a	0.30b–e	3.9cd	. . .
SC203	18ab	0.54a	8.5a	. . .	5.8a–c	0.35ab	7.0a	. . .
SC215	15cd	0.31d–f	4.4gh	. . .	6.3a–c	0.20g	3.0d–f	. . .
SC242	16bc	0.22fg	3.3hi	. . .	4.2bc	0.24e–g	2.4f	. . .
SC252	19ab	0.20g	3.0i	. . .	4.1c	0.22gf	2.3f	. . .
SC334	15cd	0.49ab	7.3bc	. . .	5.3a–c	0.31a–d	5.0b	. . .
SC367	14c–e	0.49ab	8.1ab	. . .	5.7a–c	0.37a	6.3a	. . .
SC460	18ab	0.33de	6.5cd	. . .	6.4a–c	0.28c–f	3.9b–d	. . .
SC543	15c–e	0.39cd	6.1de	. . .	5.6a–c	0.26c–g	3.8cd	. . .
SC655	16bc	0.31d–f	5.1e–g	. . .	6.6ab	0.25c–g	4.0b–d	. . .
SC723	14de	0.35c–e	6.2de	. . .	4.7a–c	0.25c–g	4.0b–d	. . .
SC760	16bc	0.43bc	5.8d–f	. . .	5.2a–c	0.27c–f	3.7c–e	. . .
SC761	15c–e	0.36c–e	6.4cd	. . .	5.8a–c	0.32a–c	4.2bc	. . .
SC835	15cd	0.21g	3.6hi	. . .	4.9a–c	0.22fg	2.7ef	. . .
SC905	13e	0.32d–f	4.9fg	. . .	5.4a–c	0.24d–g	3.6c–e	. . .
SC949	16bc	0.28e–g	4.2gh	. . .	4.8a–c	0.25d–g	2.9d–f	. . .

[a] Hardness measured as % vitreous endosperm from camera-lucida drawings of longitudinally dissected seeds. Diameter measured longitudinally with a caliper. Weight measured with an analytical balance. Density measured with a pycnometer. SKWCS hardness relative scale based on soft wheat = 50, hard wheat = 100.

[b] Means in a column followed by the same letter are not significantly different at $P = 0.05$ using Duncan's mean separation test.

ranking seed diameter per se (see rank correlations below), the scaling error was greatly magnified (cubed) when seed volume was calculated (volume = $4/3\pi r^3$), and resulted in roughly a threefold, but inconsistent overestimation of seed density (Table I).

Sample standard deviations for SKWCS and traditional laboratory measurements of seed diameter and weight were equivalent. When expressed as a percent of the sample mean, sample standard deviations for SKWCS hardness were approximately double those of percent vitreousness.

Simple correlations between SKWCS and traditional laboratory measurements of individual sample seed hardness, diameter, and weight were high (r = 0.75, 0.80. and 0.97, respectively) and significant at P = 0.05. Rank correlations were nearly as high (r = 0.68, 0.81, and 0.97, respectively) and significant at P = 0.05. Since SKWCS density estimates were known to be in error, correlations of those estimates with pycnometer measurements of density are not reported.

Simple correlations between SKWCS and traditional laboratory estimates of individual sample standard deviation for seed diameter and weight were r = 0.56 and r = 0.79 respectively, and significant at P = 0.05. Rank correlations were very similar r = 0.61 and r = 0.77 respectively, and significant at P = 0.05. Neither simple or rank correlations were significant for SKWCS hardness individual sample standard deviations when correlated with percent vitreousness individual sample standard deviations.

The somewhat lower correlation of SKWCS hardness mean values with percent vitreousness, and lack of correlation of individual sample standard deviations for these two hardness measures, do not detract from the application of SKWCS hardness data. Although percent vitreousness is commonly used as an indication of hardness (Gunasekaran et al 1988, Hallgren and Murty 1983, Louis-Alexandre et al 1991), it is not a direct measure of physical hardness. The SKWCS hardness score is derived from the physical force required to fracture the seed. This is a direct measure of physical hardness that is directly relevant to treatments such as dry rolling that accomplish similar physical fracturing of sorghum grain. Lack of correlation of the SKWCS hardness and percent vitreousness standard deviations would be expected because of the differences in the analytical approach.

Operation of the various components of the SKWCS with sorghum seeds was essentially trouble free with one exception. The prototype individual seed pickup mechanism was designed for elliptical shaped seed (wheat) rather than ovate to spherical shaped seed such as sorghum. Double sampling events (two seeds measured as one) did occur in nearly every sample measured. These double sampling events were easily detected by examination of the data, and software limits could be set to exclude such double sampling events from the data set. If broken seeds were not removed before measurement of the grain, errors in hardness and size values also occurred. These errors could usually be detected by examination of the data, and eliminated by adjustment of software limits. However, inspection and hand cleaning of samples is highly recommended before characterization of sorghum seed using this system.

CONCLUSIONS

Based on our results, SKWCS technology can be successfully used to measure sorghum seed hardness and weight, and to rank seed hardness, diameter, and weight. Compared to most systems used to measure these traits, it is more rapid and has the added advantage of quantifying the variation present in a sample as well as determining the mean value of the sample. Caution must be used in sample cleaning and in examination or delimiting the individual seed data to prevent outliers from events such as double sampling or cracked seeds from being included in the data set.

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