NOTE

A Method for Quantitatively Measuring Vitreous Endosperm Area in Sectioned Sorghum Grain[1]

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ABSTRACT

The relative proportion of corneous to floury endosperm in a sorghum kernel is referred to as endosperm texture. The endosperm texture of the grain has been related to storage potential, processing characteristics, and cooking properties (Rooney and Miller 1982). Insects more readily attack a soft, floury endosperm grain than a corneous sorghum. Munck et al (1982) concluded that the corneousness or hardness of sorghum grain determines the flour color and yield of traditional-decorticated sorghum products, as well as the products from industrial abrasive dehullers. The particle size of a flour (Kirleis and Crosby 1982) and other important factors related to food quality of sorghum are also influenced by endosperm texture (Scheuring et al 1982; Murty et al 1982a, b; Cagampang et al 1982). Endosperm texture is commonly determined by subjectively rating longitudinal half-kernel sections on a scale of one to five, with one being a kernel that contains very little floury endosperm and five being one that is essentially all floury (Maxson et al 1971; Kapasi-Kakama 1976; Murty and House 1980). The reliability of this determination depends on the skill and experience of the individual making the rating. Recently, Munck et al (1982) used an image analyzer to measure the percentage of soft endosperm on transverse sections of sorghum grain, but they did not give the error associated with their sectioning and measuring techniques. We were interested in determining the relationship among sorghum grain endosperm texture and several other parameters used to measure sorghum hardness. An image analyzer was not available in our laboratory, so we developed a simple technique for sectioning and quantitatively measuring the corneous and floury endosperm areas of sorghum grain. The procedures used for embedding, sectioning, and determining the percent vitreousness in sorghum grain are described.

MATERIALS AND METHODS

Grain from the sorghum cultivars IS0452, 954130, 850649, 954062, and IS1461, grown at the Purdue University Agronomy Farm, near West Lafayette, IN, during the 1980 crop year were used in this study. Grain from each cultivar was cleaned on a Carter dockage tester (CEA-Carter-Day Co., Minneapolis, MN), and the remaining broken and shriveled kernels were manually removed. One-kg grain samples from each cultivar were equilibrated in the laboratory at ambient conditions to a moisture content of 10.5 ± 0.5% before testing. Subsampling was done on a seed splitter (Seedburo Equipment Co., Chicago, IL) to obtain a representative 100-g sample. Then, each 100-g sample was manually quartered and opposite corners combined until 30-kernel samples were obtained.

Percentage of Vitreousness

Thirty kernels were placed in a 2.54-cm plastic cup, with the germ tip to penicle axis in a horizontal position. A firm setting, low viscosity epoxy resin (Spurr 1969) was slowly added to a depth of approximately 1 cm and allowed to polymerize overnight at 65° C. Longitudinal cross sections parallel to the germ face of the embedded kernels (Fig. 1B) were prepared by sanding with 60- and 100-grit sandpaper until the largest area of the floury endosperm was brought to the surface. When the cup is held close to a light, the largest area of the floury endosperm can be seen; we determined it to be the approximate center of the kernel. A small part of the germ was exposed also when the kernels were correctly positioned and sectioned. Sectioned kernels with no exposed germ or obviously out of position were not measured.

The image of the total and vitreous endosperm was traced under a light microscope (Wild M20-35207) with an external light source using a 15×W eyepiece, 2.5×/0.08 objective, and 1.25× camera lucida. A schematic illustration of the apparatus is shown in Fig. 2. The actual magnification of the tracing was 27.5 times the kernel size. The traced endosperm areas were then measured with a planimeter. In one experiment, the floury endosperm area was also determined, to establish the error associated with endosperm area measurements. The percentage of vitreousness was calculated by dividing the area of the vitreous endosperm by the area of total endosperm times 100.

Experimental Design

To determine the error resulting from the percentage of vitreousness measurement technique, a single sectioned kernel from cultivars IS0452, 954130, and 850649 was traced five times and measured. Cultivars IS0452, 954130, and 850649 were selected to represent a small vitreous kernel, a large kernel with intermediate vitreousness, and an intermediate size kernel with a floury endosperm, respectively.

To determine the error resulting from the sectioning technique, two 30-kernel sections or two cups of cultivars 954062, 954130, IS1461, and 850649 were measured. These cultivars were selected to represent a range of endosperm texture corresponding to 62, 47, 26, and 10% vitreousness, respectively. The model shown in Table I for the expected mean square from the analysis of variance was used to calculate the source of variance. The repeatability of the sectioning technique was expressed as the fraction of total variation found among the kernel measurements (Baker and Campbell 1971).

RESULTS AND DISCUSSION

Figure 1 shows the three types of sorghum kernel cross sections used to measure endosperm texture. Two sections are longitudinal cuts and can be characterized by using the germ face as a reference point. The germ face is defined as the exposed surface of the germ located at the base of the kernel. A longitudinal cross-section through the germ face is shown in Fig. 1A. This is the type of section commonly made when sorghum endosperm texture is subjectively rated on a one to five scale (Maxson et al 1971). If the longitudinal section is made parallel to the germ face through the largest dimension of floury endosperm, as indicated by the Y-axis in Fig. 1A, the cross-section shown in Fig. 1B is obtained. This was the sectioning technique we used. If the section is made through the

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TABLE I
Expected Mean Square, Variance Components, and Repeatabilities from the ANOVA for Testing the Repeatability of the Percentage of Vitreousness Method[a]

Source	Expected MS[b]	Variance	R[c]
Between cultivars	σ^2K/cup + $n\sigma^2$ cup/cult. + $2n\sigma^2$ cult.	438.46	0.830
Cup/cultivars	σ^2K/cup + $n\sigma^2$ cup/cult.	4.36	0.008
Kernels/cup	σ^2K/cup	85.26	0.162
Total		528.08	1.000

[a] Calculated from ANOVA shown in Table IV.
[b] n = 27.125 (weighed average number of kernels measured per cultivar).
[c] Repeatability (R) = σ^2/σ^2 total.

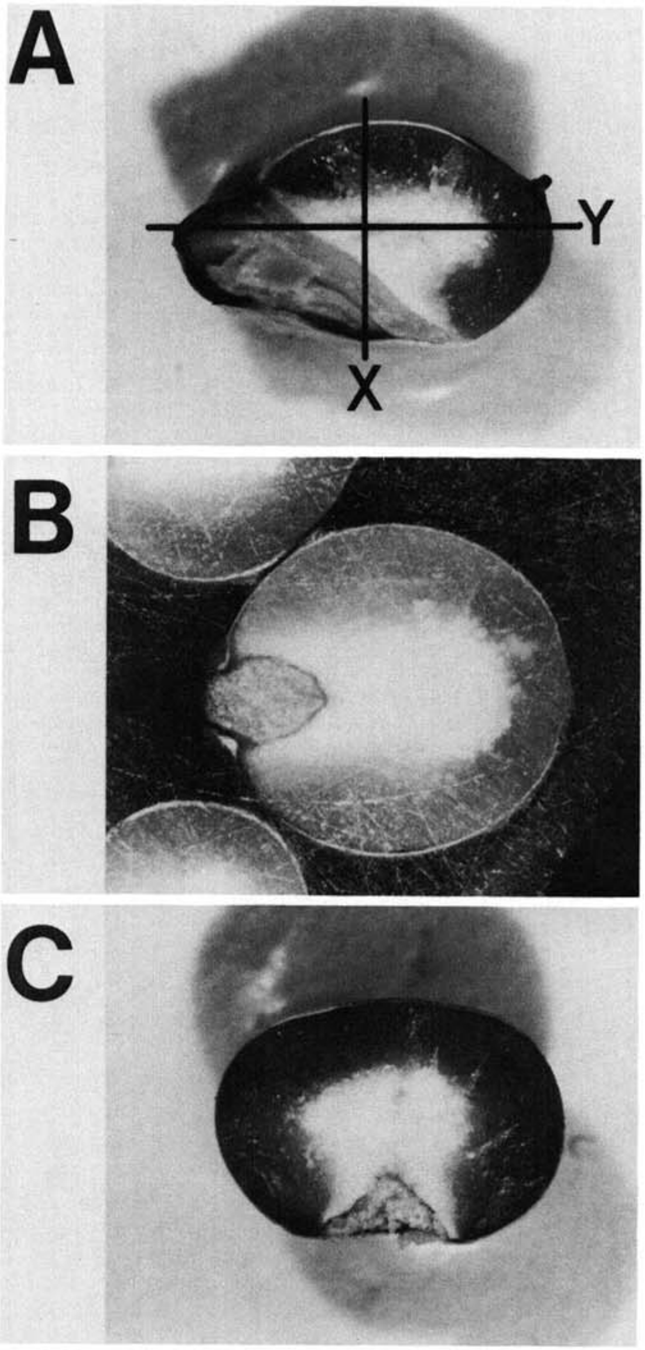

Fig. 1. Cross-sectional views of a sorghum kernel: A, a longitudinal cross-section through the germ face, with the Y- and X-axes showing the position of sectioning to obtain B and C, respectively; B, a longitudinal cross-section parallel to the germ face; C, a transverse cross-section.

TABLE II
Error Due to Measurement by the Percentage of Vitreousness Method[a]

Cultivar	Endosperm Area Units[b] Total	Vitreous	Floury	Vitreousness (%)
IS0452	1248 ± 11	784 ± 6	464 ± 8	62.8 ± 0.40
954130	1524 ± 12	774 ± 12	750 ± 8	50.8 ± 0.52
850649	1382 ± 6	121 ± 9	1261 ± 12	8.8 ± 0.66
Overall standard deviation[c]	10.3	9.3	9.2	0.54
Coefficient of variability (%)[c]	0.74	1.67	1.11	0.88

[a] Average of five tracings of a sectioned kernel ± one standard deviation.
[b] 1,000 units = 64.52 cm^2.
[c] From one-way ANOVA across the three cultivars.

TABLE III
Mean and Number of Observations Measured from Two 30-kernel Sections for Testing the Repeatability of the Percentage of Vitreousness Method

	Cup 1		Cup 2	
Cultivar	Mean % Vitreousness	Number of Observations	Mean % Vitreousness	Number of Observations
954062	58.3	28	62.5	28
954130	45.1	27	46.8	27
IS1461	28.8	25	26.1	27
850649	15.6	27	10.0	28

TABLE IV
ANOVA for Testing the Repeatability of the Percentage of Vitreousness Method

Source	DF	SS	MS	F-Ratio
Between cultivars	3	71,969.36	23,989.79	281.37[a]
Cups/cultivars	4	814.17	203.54	2.39
Kernels/cup	209	17,819.85	85.26	...
Total	216	90,603.38	...	...

[a] Significant at $P = 0.01$.

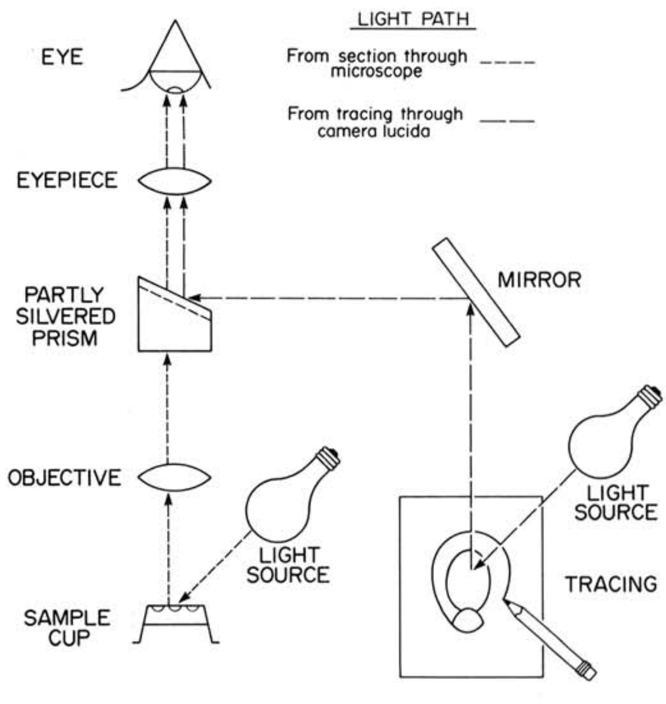

Fig. 2. A schematic diagram of a microscope equipped with camera lucida for tracing endosperm images from a sorghum kernel cross-section.

median of the longest dimension of the kernel, as shown by the X-axis in Fig. 1A, the transverse cross-section shown in Fig. 1C is obtained. This was the section used by Munck et al (1982) for determining the percentage of soft endosperm by image analysis.

To determine the variability associated with the percentage of vitreousness measurement technique, the overall standard deviation and coefficient of variability were calculated from the one-way analysis of variance. The area measurements of total, vitreous, and floury endosperm had almost the same overall standard deviation, but the coefficient of variability was smallest for the total endosperm measurement (Table II). The total endosperm area measurements of the small (IS0452), intermediate (850649), and large (954130) kernels were reflected by the total endosperm area measurement (Table II).

The difficulty in distinguishing between vitreous and floury endosperm parts appeared to increase as the endosperm texture became more floury. This was indicated by the standard deviations of the percentage of vitreousness measurement, which also increased as kernel vitreousness decreased (Table II). However, the overall coefficient of variability was less than 1% for the percentage of vitreousness measurements, indicating that the errors involved in tracing the endosperm parts, measuring the areas, and calculating the percentage of vitreousness were much smaller than the percentage of vitreousness of the kernels. Therefore, the measurement technique used was very reliable.

Because sectioning 30 kernels at once with sandpaper is a rather crude technique, we tested the repeatability of making kernel cross-sections. Table III shows the mean percentage of vitreousness and number of observations, taken from two 30-kernel sections or two cups per cultivar. As indicated by the number of observations, three of the 30 kernels were not measured, because they had no exposed germ or were out of position. The analysis of variance indicates that differences between cultivars were highly significant ($P = 0.01$) but differences between cups in a cultivar were not significantly different (Table IV). Repeatabilities show that 83, 16, and less than 1% of the total variance was due to between-cultivars, between-kernels in a cup, and between cups in a cultivar variations, respectively (Table I). Because the variation between cups in a cultivar was so small, compared to the variations between kernels and between cultivars, the repeatability of the crude sectioning technique used was very reliable.

CONCLUSION

The percentage of vitreousness method is a sensitive and reliable research tool for measuring sorghum grain endosperm texture. Not only is this method more objective than the subjective rating of endosperm texture used by others (Maxson et al 1971; Kapasi-Kakama 1976; Murty and House 1980), but it can also be used to quantitatively measure grain endosperm texture without using an image analyzer.

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