Quantitative Estimation of Corn Endosperm Vitreosity by Video Image Analysis

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ABSTRACT

Vitreosity, or hardness, is an important grain quality factor for corn. An increasing amount of research is aimed at understanding the genetic and biochemical basis of vitreosity and at improving vitreosity of high-lysine (*opaque*-2) corn through breeding strategies. Previous methods for quantifying vitreosity were destructive, subjective, or required sophisticated equipment and expertise. This study evaluated a simple video image analysis procedure for quantifying vitreosity and determined how various processing steps affected the results. Kernels were surrounded with modeling clay and viewed on a light box with a monochrome video camera. The video signal was captured to a personal computer and analyzed with commercially available hardware and software. A segregating F_2 population from a cross of vitreous Pool 29 QPM × nonvitreous B73o_2 was classified visually into 10% steps of vitreosity. High correlations were observed between visual classification and average grayscale values of captured video images at all stages of processing. Grayscale value was inversely proportional to kernel thickness. Removing image background and eliminating a segment corresponding to the embryo area increased the average grayscale range and resolution, but adjustment for kernel thickness did not substantially improve the correlation. This method allows researchers and breeders to quantify vitreosity of corn and other cereal grains on a continuous scale with readily available equipment and expertise and overcomes the problems of subjectivity, destructiveness, and complexity associated with other approaches.

The desirable grain quality attributes associated with corn endosperm vitreosity, or hardness, are well known and have justified considerable research on ways to identify and increase this factor. Hard, vitreous kernels have better nutritional, dry milling, breakage resistance, and pathogen resistance qualities than soft, opaque kernels (Gunasekaran et al 1988, Kniep and Mason 1989, Louis-Alexandre et al 1991). Considerable progress has been made in overcoming the soft endosperm characteristic of high-lysine (*opaque*-2) corn through genetic modification of endosperm texture (Mertz 1992).

Methods for evaluating the vitreosity of corn and sorghum kernels include both nondestructive and destructive approaches. Density determination by ethanol displacement (Kniep and Mason 1989, Lopes and Larkins 1991) or floatation on salt solutions (Wichser 1961) are nondestructive. Various milling and grinding techniques used as indicators of hardness (Pomeranz 1986, Fox et al 1992) are destructive. Although density and grinding resistance are correlated with vitreosity, these approaches are not based on the direct visualization of the vitreous endosperm.

Corn and sorghum vitreosity have been measured directly by sectioning or bisecting the grain, followed by quantification of floury versus vitreous endosperm by using camera lucida drawings (Kirleis et al 1984), enlarged photographs (Louis-Alexandre et al 1991), or video image analysis (Hallgren and Murty 1983, Gunasekaran et al 1988). Although this approach is the most specific, in the sense that internal endosperm tissue is viewed directly, it is relatively labor-intensive and destructive.

Nondestructive video image analysis of vitreosity of wheat has been reported (Sapirstein and Bushuk 1989). Images of transilluminated bulk samples of wheat were obtained with a monochrome charge coupled device camera connected to a personal computer by an image frame grabber board. Images were analyzed by obtaining histograms of pixel intensities. The proportion of hard, vitreous kernels based on this measurement correlated well with visually determined proportions. Corn kernel hardness classification by video image analysis of reflected light color from individual kernels was reported by Liao et al (1991). However, most researchers lack the sophisticated equipment and the specialized knowledge required to develop the complex and unique data processing algorithms applicable to their particular samples.

Although this technology may be required for automatic, rapid hardness classification, such as in a commercial processing stream, many research situations involve a finite, smaller number of samples, making automation and rapidity less important.

The relatively sophisticated means of quantifying vitreosity described above ordinarily are not readily available to corn breeders, geneticists, or millers who use the crop. Consequently, subjective evaluation based on candling (viewing backlit kernels on a ground glass screen or light box) has been used to identify vitreous phenotypes (Paez et al 1969, Bauman 1971). Kernels are visually scored and assigned to arbitrary, discontinuous classes according to the ratio of vitreous to floury endosperm (Gupta et al 1979, Ortega and Bates 1983, National Research Council 1988). This technique was used to quantify chalkiness in rice grains by visually assigning grains into 10 classes (Schaeffer et al 1986).

In our experience, visual classification of kernel vitreosity on a light box is highly subjective and not very reproducible. The method intrinsically depends on the skill, experience, and visual acuity of the rater, factors which necessarily vary among investigators. To circumvent these problems, we developed a direct, nondestructive method for quantifying vitreosity in corn kernels on a continuous scale that possesses the significant advantage of universal applicability with commercially available equipment. We also investigated the contribution of several of the processing steps to the resulting data.

MATERIALS AND METHODS

Corn Samples

To obtain corn kernels exhibiting a range of vitreosity from 0 to 100%, we used an F_2 population from a cross of a vitreous, modified o_2 line (Pool 29 QPM) with a nonvitreous *opaque*-2 inbred line (B73o_2), obtained from R. Bergquist, Pfister Hybrid Corn Co., El Paso, IL. To examine 100% vitreous kernels of various thicknesses, kernels were selected from a sample of Pool 29 QPM.

Equipment

Kernels were viewed on a standard light box with a white plastic top and 14-W fluorescent bulb (Logan Electrical Co., Chicago, IL) with a Cohu model 4815, monochrome CCD video camera fitted with a Precision Optics model 6XZMA TV lens (12.5–75 mm used here at 75 mm, $f1.8$, manual zoom-focus-aperture) and a 30-mm Cosmicar lens extension tube (Cohu, Inc., San Diego, CA). The camera provided a linear output signal ($\gamma = 1.0$). The video signal was transferred by a DT2855 Quick Capture monochrome frame grabber board (Data Translation, Marlboro, MA) installed in an IBM-compatible personal computer (Intel 80486–33 mHz processor) and connected to a Panasonic TR124MA monochrome external monitor. The DT2855 board provided a resolu-

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tion of 640 × 480 square pixels and 256 gray levels. Images were analyzed with Global Lab Image software (Data Translation) operating under Microsoft Windows 3.1.

Procedure

Kernels were placed on a small plate of clear glass with the embryo facing down and surrounded with opaque modeling clay. This was most easily accomplished by rolling a small piece of clay into a cylinder (60 mm × 2 mm) and wrapping this around the kernel, starting from the tip cap. After pinching the clay together at the kernel base, a second cylinder (5 mm diameter) was applied, and the clay was adjusted to eliminate light leaks around the kernel. The light box was covered with opaque black paper, except for a 15-mm hole centered under the camera. For viewing, the kernel mounted on the glass plate was placed over the hole and under the lens, which was 52 mm above the light box surface. Under these conditions, the kernel occupied about two thirds of the image field. The resolution was 0.001 mm² per pixel (1 mm = 32 pixels).

A sliding black paper cylinder was fitted to the camera and lens so that, when lowered, all room light was excluded, and only light transmitted through the kernel entered the camera. The live image obtained at $f5.6$ was frozen and saved to the computer disk as a 256 grayscale image file (8-bit, TIF format) (Fig. 1a). Then the paper cylinder was raised to allow room lighting to illuminate the top kernel surface, and the aperture was opened fully. A second TIF file representing the toplit image was saved (Fig. 1b). The toplit image was remapped with the image editing software so that pixels above grayscale 100 were changed to 0 (black) and pixels below 100 were changed to 255 (white). The threshold of 100 was selected because it most closely corresponded to grayscale values occurring within the abrupt transition between the dark background and the bright toplit kernel. The resulting silhouette image (Fig. 1c) was subtracted from the original file (Fig. 1a) to create an image consisting of the kernel surrounded by black (Fig. 1d). This procedure allowed accurate detection of the kernel area using the software's threshold function to select objects with pixels of 20–255 grayscale. (All pixels within the kernel area were greater than grayscale 20.) To eliminate most of the embryo area from the analysis, the drawing function was used to edit onto the image a series of lines (grayscale 16): two parallel lines delineating kernel width, a perpendicular line at the kernel base, and two diagonal lines from the center of the kernel to the intersections of the first three lines (Fig. 1e). The center of the kernel is defined here as the point midway between the two lines delineating kernel width and midway between the line delineating the kernel base and the top of the kernel. The triangular kernel area thus delineated corresponded to the portion of the image most 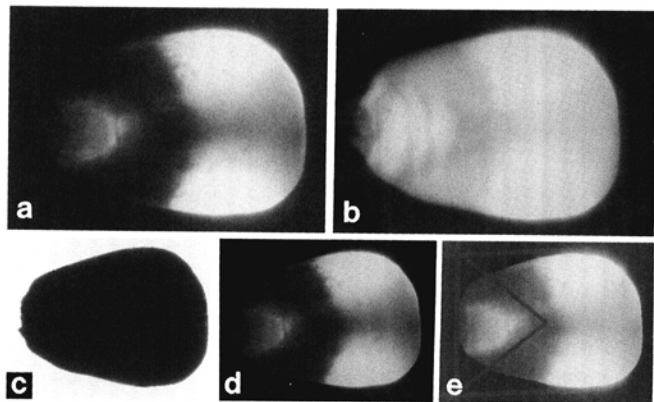affected by the presence of the embryo. This geometric approach allowed objective elimination of most pixels corresponding to the embryo zone without requiring determination of embryo areas for each kernel. The average grayscale value of the kernel, minus the embryo portion, was calculated by the particle function of the software.

To estimate kernel thickness, the left and right sides of the upper endosperm were measured with a caliper micrometer to the nearest 0.01 mm, and the values averaged. As expected, light transmittance through 100% vitreous kernels was inversely related to kernel thickness (Fig. 2). The slope of the regression line revealed that, for each millimeter of departure from the mean of 4.5 mm, the grayscale varied by 47, or 36.5%. This relationship was used to correct grayscale values of other kernels. The mean thickness of all kernels in the sample was determined, and the departure from the mean for each kernel was calculated. For each millimeter of departure, the grayscale was adjusted up or down by 36.5% to obtain the value adjusted for thickness.

To demonstrate the grayscale image obtainable from other cereal grains, vitreous sorghum, wheat, rye, barley, and oat grains were prepared as described above, with the crease facing down, except that the camera f-stop was adjusted in each case to obtain signal saturation in the brightest areas.

RESULTS AND DISCUSSION

The camera settings were adjusted so that opaque kernels (0% vitreous) gave a barely visible image (grayscale of about 20), and vitreous kernels (100%) just began to saturate the detector (grayscale 255) in the most translucent areas. These settings were identical for all three genotypes tested. When kernels were placed with the embryo facing down, light scattering through the vitreous endosperm emerged from behind the embryo, causing the embryo to appear as a diffuse shadow. In contrast, when the embryo was facing up, it was clearly delineated as an opaque region, but a large portion of the endosperm was thus obliterated. Although it was possible to obtain images without the modeling clay masking procedure, the extreme brightness of the light box background overwhelmed the sensitivity of the CCD camera and resulted in a silhouette image of the kernel. Restricting the image to transmitted light by masking with clay allowed a much wider range of grayscales to be visualized, and it eliminated camera artifacts, such as erosion and dilation. Patterns representing the internal distribution of opaque regions were obvious. The image grayscale was very sensitive to the camera aperture setting, which could be accurately fixed with a tactile detent, but it was not affected substantially by variations in focus. The average grayscale of a single kernel that was masked, viewed, and analyzed 10 times, was 118 ± 8 (±7%), with a standard error of 1.7 (1.4%). After the initial setup and some practice, it was possible to prepare a kernel, capture both backlit and toplit images, and measure
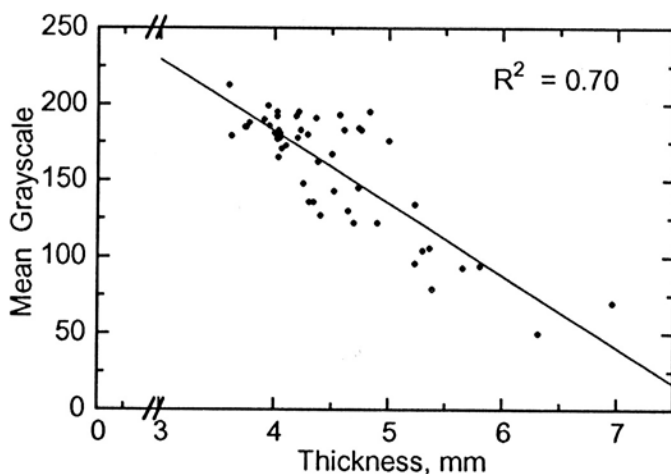
Fig. 1. Video images of a corn kernel. **a,** unprocessed image of transmitted light of kernel surrounded with modeling clay; **b,** image of kernel with both transmitted light and top lighting; **c,** remapped silhouette image of **b**; **d,** transmitted light image with background blackened by subtracting image **c** from image **a**; **e,** image **d** with five lines added to delineate a triangle corresponding approximately to the embryo area.

Fig. 2. Relationship between kernel thickness and mean grayscale value for 50 Pool 29 QPM 100% vitreous kernels.

kernel thickness within 2 min. Image processing, including editing operations and file manipulation, required an additional 2 min. The minimum time for image analysis would depend on the particular computer configuration, hard drive type, and use of keyboard macros.

To evaluate the efficacy of the method, we chose to compare the grayscale values with visually estimated percent vitreous scores because it was the most relevant nondestructive method and a consensus of skilled raters was available. From a segregating population of Pool 29 QPM $\times$ B73o_2, a set of 110 kernels was selected and visually assigned into 11 classes, 0–100% vitreous in 10% steps (Fig. 3). To determine the contribution of the various processing operations on the average grayscale, values were obtained after no processing (Fig. 4a), after eliminating the dark background (Fig. 4b), after eliminating the embryo triangle with the five-line editing procedure (Fig. 4c), and after adjusting for kernel thickness as described above (Fig. 4d). Although a relationship between the visual classification and the grayscale value could be discerned with the unaltered files (Fig. 4a), the range of grayscale values was only 29. The background removal steps extended the grayscale range to 125 (Fig. 4b). Removing the embryo triangle, which usually contained the darkest pixels,

increased the range further to 157 (Fig. 4c). Adjusting for kernel thickness did not affect the range of grayscale values (Fig. 4d). The coefficient of determination (r^2) increased from 0.85 to 0.92 as processing steps were added. A slightly sigmoidal tendency to the plots was observed, in that the increments in grayscale from 0 to 20% and from 80 to 100% were smaller than from 40 to 60%. This indicates that the subjective, visual classification of kernels into the 10% steps was not as reliable as the objective, machine vision determination. A comparison between means for each visual rating class (Table I) shows that the overlapping of classes was reduced by all image editing steps except the kernel thickness adjustment. Only a few very thick (round) or thin (flat) kernels were affected substantially by the thickness adjustment, and the grayscale variation within classes apparently exceeded the variation attributable to thickness in most cases. If more than 10 kernels per class were evaluated, or if the sample was more heterogeneous, the thickness adjustment might have made a significant improvement.

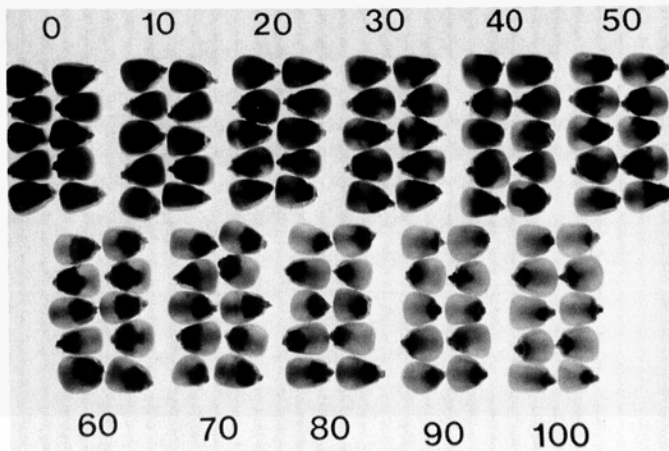The applicability of this method to other cereals was demon-

Fig. 3. Light box view of 110 kernels from an F$_2$ population of the cross Pool 29 QPM $\times$ B73o_2 visually separated into 10% vitreosity classes.

TABLE I
Mean Grayscale Values of Images of Corn Kernels from Visually Rated Classes Obtained at Four Processing Levels[a]

Percent Vitreosity Class	Mean Grayscale Value[b]			
	Unprocessed Input File	Background Removed	Embryo Removed	Adjusted for Thickness
0	24.4 a[c]	28.5 a	26.3 a	26.3 a
10	25.4 a	31.3 a	31.8 ab	31.8 ab
20	26.9 a	40.2 ab	44.3 bc	43.7 bc
30	27.4 a	50.1 b	55.6 c	56.5 c
40	31.2 b	67.2 c	79.3 b	79.7 d
50	35.2 c	81.9 d	100.5 e	99.9 e
60	37.8 c	95.2 e	119.9 f	118.2 f
70	38.2 c	100.4 e	122.2 f	122.6 f
80	45.5 d	126.8 f	153.5 g	149.0 g
90	51.2 e	143.6 g	176.2 h	179.2 h
100	53.0 e	153.0 g	183.7 h	182.8 h

[a] Kernels selected from an F$_2$ population resulting from the cross Pool 29 QPM (vitreous) $\times$ B73o_2 (opaque).
[b] Each value represents the average of 10 kernels.
[c] Means within columns followed by the same letter are not significantly different ($P = 0.05$) according to the LSD test.
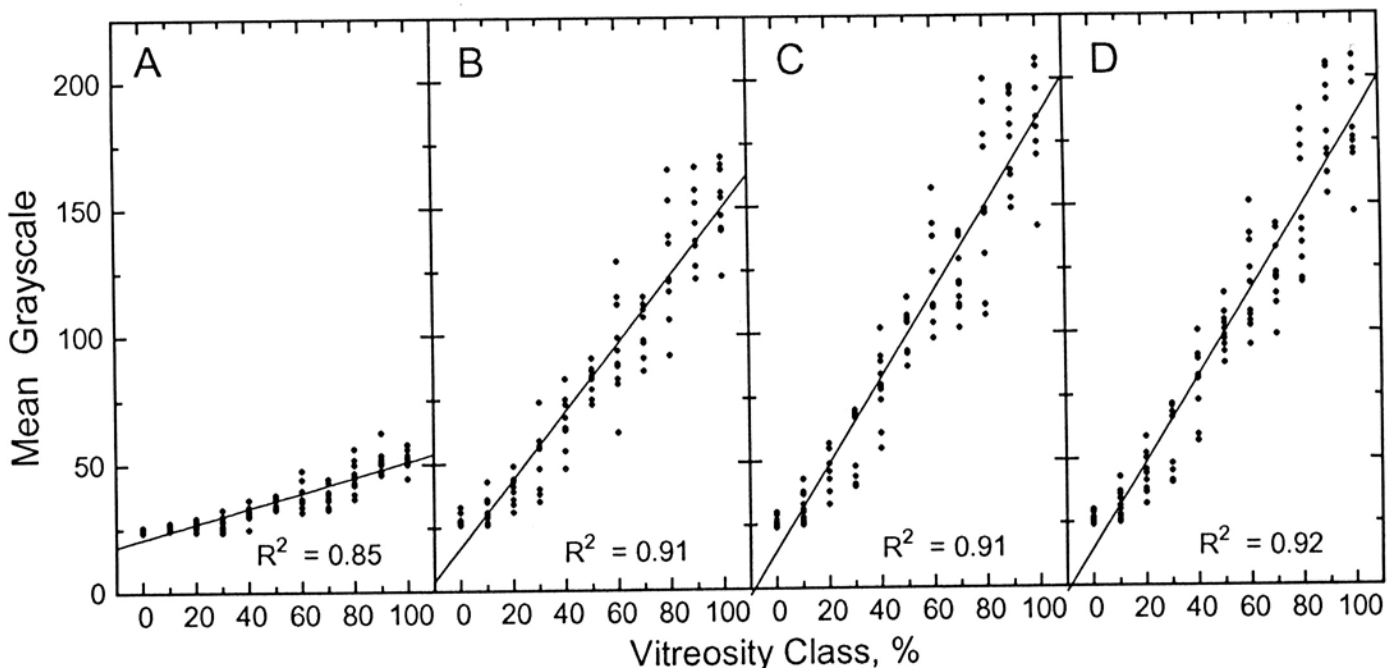

Fig. 4. Scatter plots of grayscale values obtained from the kernels shown in Fig. 3. **A,** unprocessed input file (as in Fig. 1a); **B,** background removed (as in Fig. 1d); **C,** embryo removed (as in Fig. 1e); **D,** values adjusted for kernel thickness as described in the text.

strated by obtaining grayscale images of light transmitted through sorghum, wheat, rye, barley, and oat grains (Fig. 5). Each species required a different *f*-stop setting on the camera aperture to optimize the image grayscale range. Barley and oats were viewed both with and without the hull. The venation pattern of the hull could be seen (Fig. 5d,f), and light absorption by hull structures superimposed grayscale variations irrelevant to endosperm vitreosity. Although unobstructed views of endosperm vitreosity could be obtained with hulled grains (Fig. 5e,g), it was not concluded whether the work of removing the hull would be justified by the improved image.

Paez et al (1968) measured light transmittance through normal and *opaque*-2 corn kernels with a fluoro-microphotometer. The light transmission values did not overlap, but kernels with intermediate vitreosity were not considered. Although a quantitative relationship between percent vitreousness and fluoro-microphotometer readings for variably vitreous kernels might be found, such an apparatus is less commonly available and more expensive than the equipment used in this study. Moreover, it would seem unnecessary to use a specific wavelength range or an ultraviolet light source. Hall and Anderson (1991) obtained a high correlation between density determined by floatation and light transmission of bulk corn samples determined with a calibrated light meter. Although that method employs the same principle as ours, the light transmission range was limited (34–52%), possibly by light passing between the numerous kernels. Also, the method is not applicable to single kernels.

Approaches for quantifying grain vitreosity vary in terms of the required skill and equipment, as well as the attainable resolution and speed. Subjective visual classification on a light box requires minimal equipment but considerable skill and experience. The resolution and speed depends on the skill level. As the sophistication and expense of the equipment is increased, the subjective input is lessened, but the technical skill required to apply the method increases dramatically with increased speed and resolution. The method described in this study does not approach the speed of measurement and analysis that would be required for routine quality control applications or screening of large numbers (thousands) of samples. These types of applications involve more expensive equipment and much more complex analysis than is described here (Sapirstein and Bushuk 1989, Liao et al 1991). Also, the faster and more complex machine vision methods being developed are applicable to a wider range of grain quality factors, such as stress crack detection in corn (Gunasekaran et al 1987) and fungal damage in soybeans (Paulsen et al 1989). Because our simple grayscale analysis measures only transmitted light, it is not complicated by many factors that might affect other methods, such as color variations, air pockets, and kernel size.

The speed and complexity of this method make it suitable for research situations where a quantitative measure of vitreosity is needed, and in particular, where populations of segregating progeny of specific crosses are being examined. Examples of such applications include basic research on the mechanism of vitreosity expression (Lopes and Larkins 1991) and breeding strategies involving recurrent selection or backcrossing (Villegas et al 1992). Our approach demonstrates a relatively simple application of machine vision involving only monochrome detection and grayscale analysis with relatively inexpensive equipment and software. An important advantage of this approach is the increasingly ubiquitous availability of personal computers and the rapid commercialization and decreasing cost of video imaging hardware and software.

CONCLUSIONS

The video image analysis technique described here provides a means of estimating the vitreosity of corn kernels by quantifying the brightness of corn kernel images obtained by viewing kernels on a light box with a television camera. The technique is nondestructive, objective, and flexible enough to accommodate different types of corn or other grains. It provides a continuous scale of translucency, in contrast to the discontinuous classes obtainable by subjective candling. The equipment required is commercially available and relatively inexpensive. It consists of components easily added to a personal computer. Although the technique is simple and rapid enough for most research applications, it has not yet been automated to the level necessary for commercial scale classification within a processing or transportation stream. The method is most appropriate for research situations requiring objective quantification of corn kernel vitreosity for biochemical, genetic, and agronomic studies.

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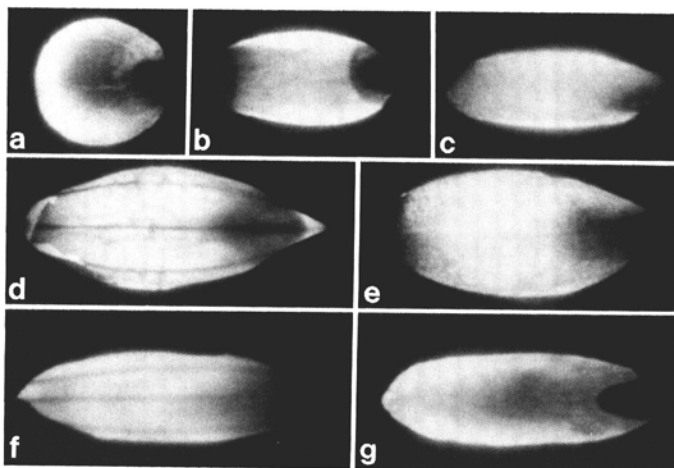Fig. 5. Video images of light transmitted through vitreous grains of sorghum (**a**), wheat (**b**), rye (**c**), barley with hull (**d**), barley without hull (**e**), oat with hull (**f**), and oat without hull (**g**).

NATIONAL RESEARCH COUNCIL. 1988. Quality Protein Maize. National Academy Press: Washington, DC.

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