Quantitative Measurement of Light Transmission through Corn Endosperm[1]

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ABSTRACT

Opaque and nonopaque corn kernels can be separated with the use of a frosted glass viewer similar in design to an X-ray viewer. For those genotypes where visual classification by the frosted glass method is difficult, measuring the transmission of light through individual kernels might be an aid in separating homozygous ($O_2O_2O_2$) and ($o_2o_2o_2$) kernels. Kernel thickness has an effect on the percent of light transmitted, thin kernels transmitting more light than thick kernels. Moisture content of kernels and effects of different days had no significant effect on percent of light transmitted. Light transmission distributions for $O_2O_2O_2$ and $o_2o_2o_2$ kernels are widely separated, and the ranges do not overlap. A mechanical-electronic device could be constructed to separate corn kernels automatically into opaque and nonopaque classes according to the percent of light transmitted.

The endosperm mutant opaque-2 has been shown by Mertz *et al.* (1) to alter markedly some of the amino acids in corn (*Zea mays* L.). The increased lysine concentration resulting from this mutant greatly improves the nutritional characteristics of corn for some mammals (2). Bressani (3) has shown corn endosperm, homozygous for the o_2 gene ($o_2o_2o_2$), to be more nutritious than normal endosperm ($O_2O_2O_2$) when consumed by children 2 to 6 years old. Likewise, Clark (4) found opaque-2 corn an adequate source of protein for adult humans.

Maize breeders are actively incorporating the opaque-2 gene into existing breeding stocks, and hybrid production is likely in the next few years (5).

Kernels homozygous for opaque-2 endosperm are phenotypically distinguishable in most genetic backgrounds. However, in certain backgrounds visual identification is difficult and exact classification is not possible (5). The object of this investigation was to determine whether a photometric device could be used for separating homozygous opaque-2 from normal kernels in segregating populations. If the method is successful, the expense of a test cross-generation could be eliminated.

MATERIALS AND METHODS

For simple separations of opaque and normal kernels, kernels are placed on a sheet of frosted glass over a light source. The opaque kernels appear dark; the nonopaque are relatively clear (Fig. 1). Our apparatus consists of a frosted-glass pane mounted over three small fluorescent tubes (GE F8T5 CW). The light intensity emitted by these tubes is similar to that

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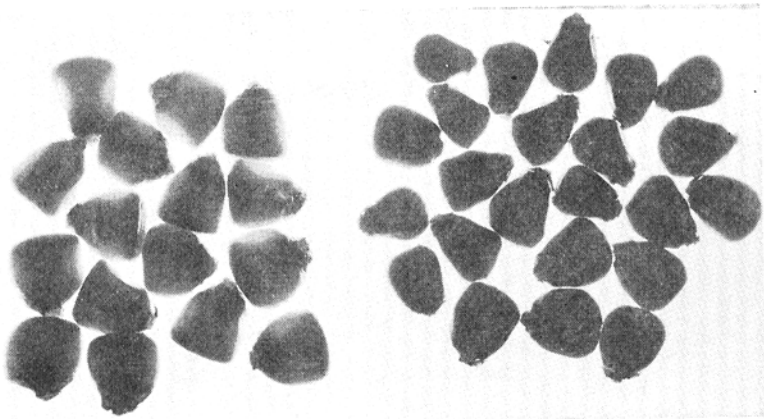

Fig. 1. Appearance of opaque and nonopaque corn kernels on a frosted-glass viewer.

employed in X-ray negative viewers. Satisfactory results can also be obtained by using the glass surface of an overhead projector.

The quantity of light transmitted through individual corn kernels was measured with an Aminco Photomultiplier Fluoro-microphotometer Model 4-7102 employing a GE F4TF/BL UV light source. Maximum transmission was obtained in the 450- to 560-mμ range. A black cardboard sample holder of measured aperture size was fitted with a modeling-clay washer, upon which individual corn kernels were mounted and sealed by pressure to prevent light leakage (Fig. 2). The holder with mounted kernel was inserted in place of the second filter, the sample cuvet being omitted (Fig. 3).

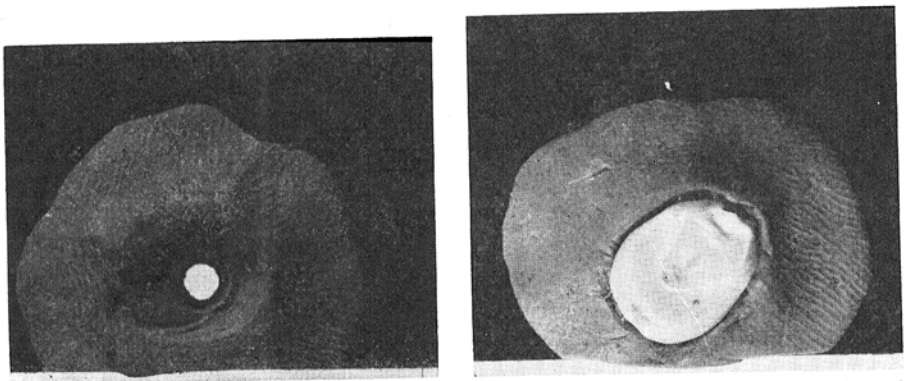

Fig. 2. Left, cardboard kernel holder with modeling-clay washer; right, kernel in position for measurement.

Holes of various diameters were punched in several holders to determine the effect of aperture size on percent of light transmitted. Aperture diameters ranged from 2.5 to 5.0 mm., which gave aperture areas ranging from 3.927 to 7.854 mm.^{2}

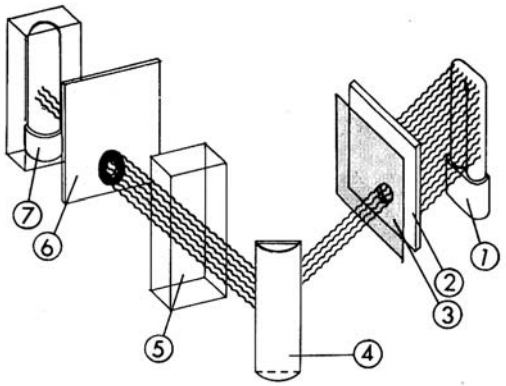

Fig. 3. Light path of the fluoromicrophotometer. 1, light source; 2, aperture plate; 3, primary filter; 4, mirror; 5, cuvet holder; 6, sample holder; 7, photomultiplier.

Ears homozygous for normal ($O_2O_2O_2$) and opaque-2 ($o_2o_2o_2$) endosperm of the inbred line MoB2 were produced in 1966. The genotypes were verified by outcrossing to known testers. Six ears chosen at random from each genotype were shelled, and kernels were sized for thickness. Thickness sizes were No. 14 (through a 15 on a 14 slotted screen) and No. 11 (through a 12 on an 11 slotted screen). One opaque-2 kernel and one normal kernel of each kernel thickness were tested at all aperture openings.

The effect of kernel moisture content on light transmission was determined by drying 10 kernels for each genotype and thickness to 3.0%. Light transmitted through the smallest aperture was measured. Tap water was added to these kernels to increase the moisture content to 21.5%. After 48 hr., measurements of light transmission were made, and after 48 hr. of air-drying to 7.5% moisture, another light transmission test was made.

The effect of different days on light transmission was determined by testing two kernel thicknesses (sizes 11 and 14) from six random ears taken from each genotype, on 4 consecutive days. These variables were studied with the use of a balanced factorial design with 10 random kernels per cell and the smallest aperture opening. A mixed model was used, assuming sizes to be a fixed variable.

RESULTS AND DISCUSSION

The frosted-glass viewer is a satisfactory means of separating opaque from nonopaque corn kernels in most genetic backgrounds. Differences between opaque and nonopaque endosperm is clearly demonstrated in Fig. 1. However, in certain inbred lines it is difficult to distinguish between opaque and nonopaque kernels. Alexander (5) reported that it was difficult to classify opaque and nonopaque kernels accurately in the inbred line R803. Corn breeders may prefer opaque-2 lines with nonopaque characteristics, as these kernels should have higher kernel weights (5). Low kernel weight, which is

reflected in lower test weight and acre yield, currently is a production disadvantage of opaque-2 corn.

The microphotometer with the UV light source as used in this study was successful in quantitatively measuring light transmission of kernels homozygous for $O_2O_2O_2$ and $o_2o_2o_2$.

The effect of aperture size on percent of light transmission is shown in

TABLE I

EFFECT OF APERTURE SIZE ON THE LIGHT TRANSMISSION OF NORMAL AND OPAQUE CORN KERNELS

	Transmission[a]			
	Size 14		Size 11	
Aperture Size	$O_2O_2O_2$	$o_2o_2o_2$	$O_2O_2O_2$	$o_2o_2o_2$
*mm.*²	%	%	%	%
3.927	0.021	0.001	0.024	0.000
4.712	.043	.004	.058	.001
5.498	.066	.005	.089	.002
6.283	.132	.005	.181	.003
7.069	.182	.004	.385	.003
7.854	0.600	0.008	0.675	0.003

[a] Average of two independent observations on the same kernel.

Table I. The homozygous $O_2O_2O_2$ kernels allowed from 0.02 to 0.67% light transmission as aperture area was increased. The increase follows a logarithmic curve. The homozygous $o_2o_2o_2$ kernels essentially had no transmission, and the small values obtained are probably due either to background noise in the photoreceptor circuit or to leakage caused by imperfect seal of the kernel to the holder. The thinner kernels (No. 11) had greater transmission at all aperture sizes.

The effect of moisture level on percent transmission of normal and opaque-2 kernels is given in the table below. Again opaque kernels gave

Moisture Content after Treatment	*Transmission (Average of 20 observations)*	
	$O_2O_2O_2$	$o_2o_2o_2$
%	%	%
3.0	0.870	0.000
21.5	0.700	0.001
7.5	0.570	0.004

essentially no light transmission; nonopaque kernels had from 0.57 to 0.87% transmission. It appears that the effect of moisture level, if any, is very slight and will not be a problem in classifying opaque and nonopaque kernels.

The effect of measuring light transmission for the same kernels at different times was investigated. The study involved 10 kernels from each kernel size (Nos. 11 and 14), taken from six random ears and representing each genotype ($O_2O_2O_2$ and $o_2o_2o_2$). Light transmission was measured on 4 consecutive days. The percent of light transmitted and the percent of lysine on a whole-kernel basis are given in Table II. No significant differences in light transmission were found among samples of the $O_2O_2O_2$ genotypes measured

on different days (Table III). This suggests that one could expect good repeatability from determinations made at different times. The thicker kernels (No. 14) again transmitted less light than the thinner ones (No. 11). The interaction of days by ears cannot be explained.

TABLE II

PERCENT LIGHT TRANSMISSION BY OPAQUE AND NONOPAQUE KERNELS, AVERAGED OVER 4 DAYS, AND THEIR LYSINE CONTENT

EAR NO.	$O.O_2O_2$			$O_2O_2O_2$		
	Transmission		Lysine Content	Transmission		Lysine Content
	Size 14	Size 11		Size 14	Size 11	
	%	%	%	%	%	%
1	0.087	0.124	0.30	0.001	0.001	0.48
2	.115	.205	.28	.001	.000	.49
3	.074	.094	.28	.000	.004	.44
4	.093	.130	.21	.009	.005	.48
5	.123	.142	.30	.005	.005	.46
6	0.118	0.188	0.24	0.012	0.003	0.49

TABLE III

ANALYSIS OF VARIANCE AND VARIANCE COMPONENTS ESTIMATES FOR PERCENT LIGHT TRANSMISSION OF HOMOZYGOUS O_2 KERNELS

SOURCE OF VARIANCE	D.F.	M.S.	VARIANCE ESTIMATE
Ears	5	0.0693**	0.00070
Sizes	1	.2471**	.00004
E × S	5	.0169	.00000
Days	3	.0132	.00000
D × E	15	.0136*	.00029
D × S	3	.0184	.00000
D × S × E	15	.0265**	.00187
Error	432	0.0078	0.00778
Total	479		

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