Characterization of Colored-Grain Sorghum Lines and Identification of High-Tannin Lines with Good Dehulling Characteristics[1]

R. D. REICHERT,[2] M. A. MWASARU,[3] and S. Z. MUKURU[4]

ABSTRACT

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Colored-grain sorghum lines were characterized with the objective of identifying lines with a high tannin content and good dehulling quality. Physical and chemical analysis of grain from 1,768 lines showed a wide variation in tannin (0–11.8%), abrasive hardness index (2.5–21.3 sec), weight (1.0–7.8 g/100 seeds), shape, and color. The majority (85%) of the lines had tannin contents of 5% or less. Five lines with greater than 10% tannin were identified. High-tannin (>1%) lines were generally slightly softer than low-tannin (<1%) colored-grain lines and much softer than noncolored lines. However, six high-tannin lines with hardness exceeding the mean of 21 noncolored lines were identified. Seventy percent of lines had a seed weight in the range of 1.0–3.0 g/100 seeds, and most had flat kernels. Twenty-four color classes could be visually distinguished. Generally, the lines within each color class varied widely in tannin content and hardness. Several light-colored lines were identified that lacked a spreader gene but contained high levels of tannin. Low but significant correlations were observed among many of the physical and chemical properties of the lines. Several high-tannin lines were identified that could be dehulled with flour extraction levels of at least 70% to produce flour low in tannin and acceptable in color.

The Genetic Resources Unit of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) considers colored-grain sorghum lines to include the following (S. Z. Mukuru, *personal communication*): red grain, without a testa layer but with a colored pericarp; brown grain, with a testa layer and colored pericarp; and white grain, with a testa but without a red or brown pericarp. By 1983, when this study was initiated, ICRISAT had collected approximately 19,925 sorghum lines, of which 4,119 were considered to be colored-grain lines. Since then, the collection has increased beyond 25,000 lines, and the proportion of colored-grain lines has increased to approximately 35%.

Many colored-grain sorghum lines contain high levels of tannins, which occur mainly in the pericarp and testa layers of the caryopsis (Earp et al 1983, Glennie 1983). Tannins protect the grain from insects (Woodhead et al 1980), birds (Bullard et al 1980), and preharvest germination (Harris et al 1970). However, high-tannin sorghums are unfortunately nutritionally inferior when compared with low-tannin types of similar composition (Chang and Fuller 1964, Dreyer and van Niekerk 1974, Jambunathan and Mertz 1973, Maxson et al 1973, Muindi and Thomke 1981, Schaffert et al 1974). It would be desirable to retain the agronomic advantages associated with production of high-tannin sorghums and devise means to overcome their deleterious nutritional effects. Methods that have been developed to deactivate the tannins include treatment or extraction of the grain with water, acids, alkalis, or formaldehyde (Armstrong et al 1974, Butler 1982, Daiber and Taylor 1982, Featherston and Rogler 1975, Mitaru et al 1983, Price et al 1979, Reichert et al 1980).

Another approach to improvement of nutritional quality of high-tannin sorghum is removal of tannin-containing layers by abrasive dehulling, particularly since these systems are now being actively introduced in Africa for use on sorghum (Eastman 1980, Cross 1985). However, investigators showed that high-tannin grain was only poorly dehulled with either low yield and a high protein loss (Chibber et al 1978), or at a reasonable yield and low throughput (Mwasaru et al 1988). The latter investigators suggested that the grain characteristics of high-tannin sorghum needed to be improved before abrasive dehulling of such cultivars could be commercialized. The objective of this study was to characterize chemical and physical features of a large population of colored-grain sorghum lines in an attempt to identify high-tannin lines that could be effectively dehulled to yield flour low in tannin and acceptable in color.

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[2]Plant Biotechnology Institute, National Research Council of Canada, 110 Gymnasium Road, Saskatoon, Saskatchewan, S7N 0W9.

[3]Kenya Industrial Research and Development Institute, P. O. Box 30650, Nairobi, Kenya.

[4]International Crops Research Institute for the Semi-Arid Tropics, Patancheru, A.P., India.

MATERIALS AND METHODS

All colored-grain sorghum lines in the ICRISAT collection (4,119) were increased in the 1982-1983 post-rainy season (November to March), and a representative sample (1,768) of the population was selected. This subset was regrown at ICRISAT, Patancheru, India, during the 1983-1984 post-rainy season. The grains were grown on red soils with row spacing of 0.75 m and row length of 4 m. There were 35–40 plants per row, giving a plant density of 18–20 plants/m^2 after thinning. The matured crop was harvested, sun-dried, and hand threshed to avoid the excessive kernel breakage caused by mechanical threshers. The moisture content (method 44-15A, AACC 1983) of 30 randomly selected samples was 10.8 ± 0.7% (standard deviation). The cleaned grain was stored in double plastic bags at 15°C. All analyses of the samples were completed within eight months of harvest.

Analytical Methods

Tannin content. Grain was ground in a Udy Cyclo-Tec mill to pass a 0.5-mm screen and analyzed (in duplicate) for tannin content by the method of Burns (1971) using reagent blanks described by Price et al (1978). The detailed procedure has been described elsewhere (Mwasaru 1985).

Abrasive hardness index (AHI). Grain hardness measurements (in duplicate) were determined using the Tangential Abrasive Dehulling Device (TADD, Venables Machine Works Ltd., Saskatoon, SK) equipped with a 12-cup sample plate and a grinding wheel (Norton Canada Inc., Hamilton, ON) with the following specifications: A36L5VBE, 10-in. diameter, 3/8-in. thick, 1-in. arbor, V-sided, 9ORD, diameter ± 0.005 in., thickness ± 0.005 in. (Reichert et al 1986). The TADD was operated at 1,450 rpm. All samples were dehulled for 4 min, and the percent kernel removed by dehulling was determined by grain depth measurement as described by Reichert et al (1986). The AHI was calculated as the time in seconds to remove 1% of the kernel as fines (Oomah et al 1981).

Seed weight. One hundred seeds (in duplicate) were counted in an electronic seed counter (The Old Company, Savage, MD) and weighed.

Seed shape. The shape of seeds was subjectively assessed on a scale of 1 to 5, where 1 indicated a spherical seed and 5 a flat one (Murty et al 1984). The average of six seeds was determined.

Seed color. Twenty-four color classes could be visually distinguished among the 1,768 lines. A representative line from each color class was analyzed using a model D25 Hunterlab color difference meter (Hunterlab Associates Laboratory, Fairfax, VA) standardized with a tile with $L = 94.7$, $a = -0.9$, and $b = 0.5$.

Selection Procedure for Identifying High-Tannin Lines with Good Dehulling Characteristics

Following the analysis of all colored-grain sorghum lines (n = 1,768) for tannin, AHI, weight, color, and shape, the lines with tannin greater than 1% and AHI greater than 11.5 sec were identified. A subset of half of these samples was dehulled in the TADD (1,750 rpm) at four dehulling times (1, 2, 4, and 6 min). For each line, the flour extraction was plotted against the flour tannin content to determine at what flour extraction the tannin content was equal to 0.5% (defined as the flour extraction [tannin]). Flour-water slurries of the whole and all dehulled flour samples of a subset of 16 of the 28 lines having a flour extraction (tannin) of at least 70% were analyzed for color using an Agtron M-500 spectrophotometer (Magnuson Engineers, San Jose, CA). Flour (2 g) was weighed into the Agtron sample cup, and distilled water was added (3 ml) to give a thin slurry. The slurry was well mixed and allowed to stand for a few minutes before a reading at 436 nm was taken. The Agtron was standardized using the 00 and 90 color standards. The flour extraction was plotted against the flour color to determine at what extraction the flour color was equal to 30 or 35% (defined as flour extraction [color-30] and flour extraction [color-35], respectively). Lines with flour extraction (color-30) and flour extraction (color-35) of at least 70% were identified.

RESULTS AND DISCUSSION

Detailed grain characteristics of each of the 1,768 colored-grain sorghum lines are contained in a thesis by Mwasaru (1985). The following discussion summarizes the results.

Grain Characteristics of Colored-Grain Sorghum

Tannin content. The distribution of tannin content among the 1,768 colored-grain sorghum lines showed a very wide range (0.0–11.8%), with the majority (85%) of the population containing less than 5% (Fig. 1). The modal class (2.0–3.0% tannin) accounted for 21.2% of the population. Most of the population (n = 1,421) contained greater than 1% tannin; these would be considered group III sorghum types (Axtell et al 1974). Other investigators have reported ranges of 0.0–6.7% (Earp et al 1981) and 0.0–7.2% (Subramaniam et al 1983) for the tannin content of sorghum using the vanillin assay. Axtell et al (1974) reported a mean tannin content of 3.4% for 172 high-tannin sorghum lines, which agrees with the mean value (3.4%) obtained for the high-tannin group in this study.

Some sorghum lines (IS 12591, 8875, 12593, 2880, and 9215) with greater than 10% tannin were identified. These lines could prove to be desirable if economic industrial uses for sorghum tannin were developed, such as in leather tanning (Seigler et al 1986). The bran fraction obtained by dehulling these lines would be an even more concentrated source of tannin.

Grain hardness. The distribution of AHI among colored-grain sorghums showed a very wide range (2.5–21.3 sec); the modal class (6.0 to 8.0 sec) accounted for 28.1% of the population (Fig. 2). The low-tannin (< 1%) group displayed a wide range of AHI (2.8–21.3 sec) and a mean of 9.9 sec, which was higher than that of either the total population (7.7 sec) or the high-tannin group (7.1 sec). The mean AHI for 21 noncolored sorghum lines was found to be 15.7 sec (range = 8.9–26.2 sec), which confirmed that noncolored lines are considerably harder than colored-grain lines. Six high-tannin lines (IS 9854, 21509, 8183, 7179, 8186, and 21045) with AHI values exceeding the mean of the noncolored lines were identified.

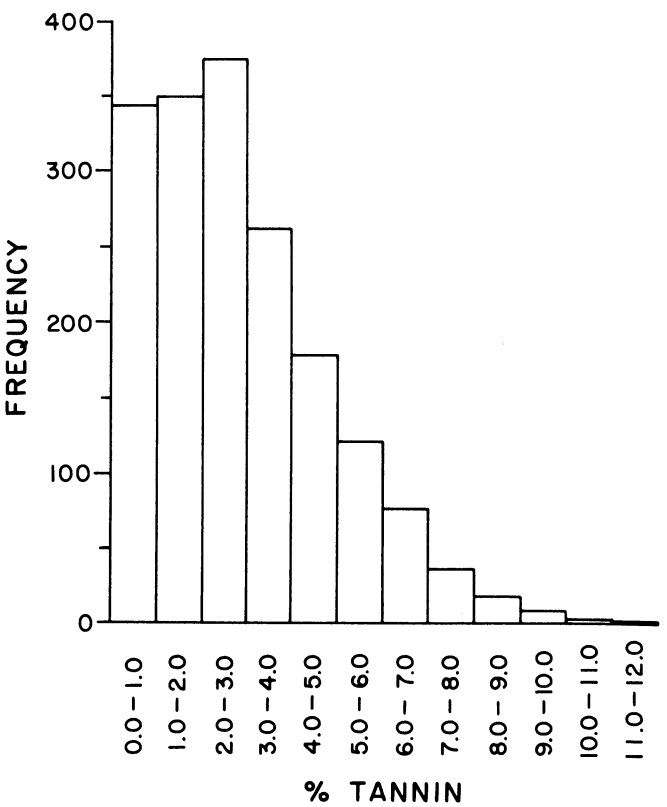

Fig. 1. Distribution of tannin among colored-grain sorghum lines (n = 1,768). The minimum, mean, and maximum tannin contents were 0.0, 2.8, and 11.8%, respectively.

Seed weight. The distribution of seed weight among colored-grain sorghums showed a wide range (1.0–7.8 g/100 seeds); the modal class (2.0 to 2.5 g/100 seeds) accounted for 29% of the population (Fig. 3). Lines with seed weights less than 3.0 g/100 seeds accounted for 70% of the population. These results are in agreement with those of Freeman (1970) who observed that the majority of sorghums had seed weights of 2.0–3.0 g/100 seeds. A wider range in seed weight was observed among the high-tannin group (1.0–7.8 g, mean = 2.7 g) than among the low-tannin group (1.5–5.3 g, mean = 3.0 g). A wide range in sorghum seed size was previously reported (2.0–7.6 g, Murty et al 1982; 2.3–5.5g, Subramaniam and Jambunathan 1982).

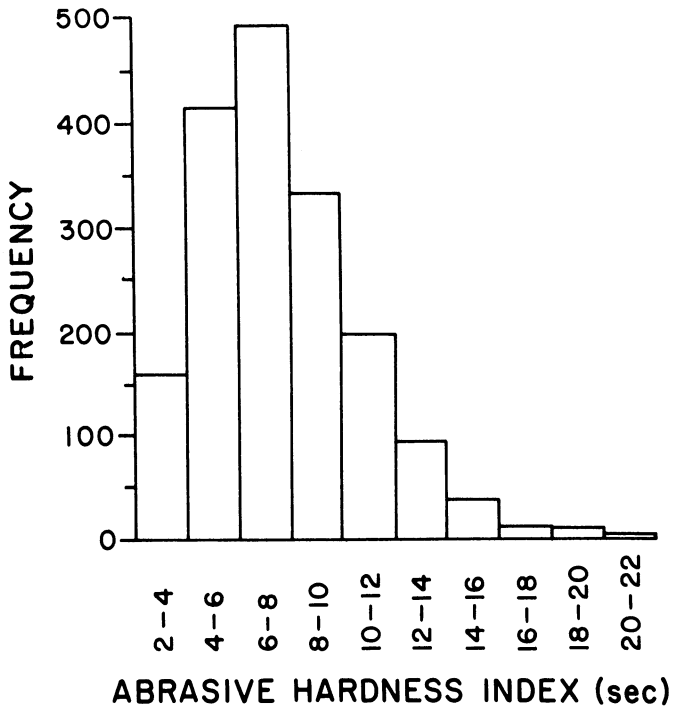

Fig. 2. Distribution of abrasive hardness index (AHI) among colored-grain sorghum lines (n = 1,768). The minimum, mean, and maximum AHI were 2.5, 7.7, and 21.3, respectively.

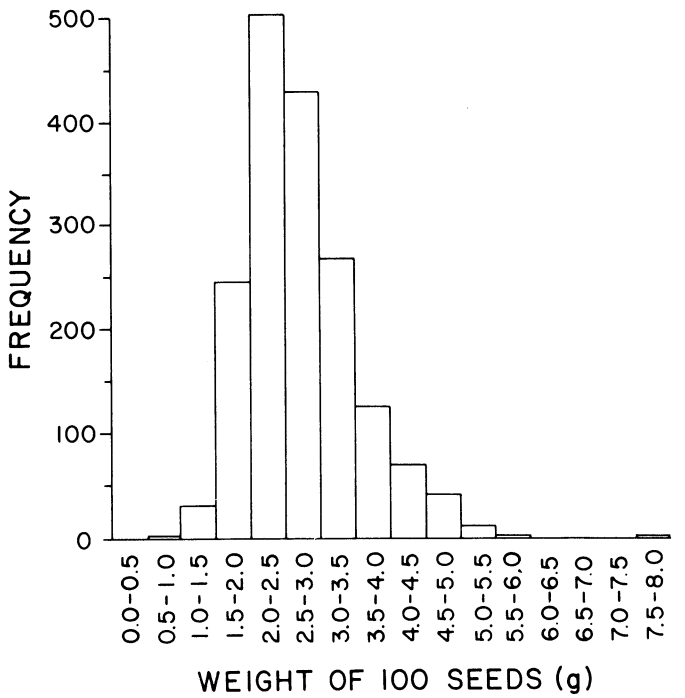

Fig. 3. Seed weight distribution among colored-grain sorghum lines (n = 1,768). The minimum, mean, and maximum seed weights were 0.96, 2.7, and 7.8 g per 100 seeds, respectively.

Seed shape. The majority of colored-grain sorghum lines were observed to have flat kernels (Fig. 4). Shape ratings of 4 and 5 were assigned to 74% of the population. Only 0.6% of the population had kernels that were considered round (shape rating = 1). The high- and low-tannin groups had mean seed shapes of 4.2 and 3.6, respectively. Murty et al (1984), using the same rating scale, determined seed shape for 25 sorghum cultivars and observed a range of 1–5 with a mean of 2.8. The high-tannin sorghum population under study appeared to have flatter kernels than either the low-tannin group or the sorghum lines studied by Murty et al (1984).

Seed color. The colored-grain sorghum lines exhibited a diversity of color ranging from light gray to dark brown, and the number of samples in each color class appeared to be randomly distributed (Table I). The largest color class (21) accounted for 13.4% of the population. Color classes were ranked on the basis of the magnitude of the L value (white = 100, black = 0), which ranged from 21.3 to 53.2. The samples exhibited a combination of yellow and red hues, as indicated by positive values of a and b. The Hunterlab L value has been used previously to describe the color of sorghum grain and sorghum food products (Iruegas et al 1982, Rooney and Murty 1982).

Generally, the lines within each color class varied widely in tannin content and AHI. However, all purple lines with white spots (color class 3) were low in tannin content (0.0–0.6%), and all lines in color classes 2, 14, 22, and 24 contained at least 1% tannin. Some intensely dark brown lines (color class 23) contained very low tannin contents. Every color class contained some lines with very low AHI values (2.5–4.7 sec).

Several sorghum lines in color classes 1, 3, and 5 lacked the brown or reddish pigmentation associated with the presence of the spreader gene (S), yet contained greater than 1% tannin. Three light gray lines (IS 7134, 9788, and 12584) in color class 1, for example, contained 2.0, 2.4, and 3.4% tannin. On the basis of the absence of the spreader gene, these lines would have been expected to contain less than 1% tannin by the vanillin assay (Earp et al 1981, Rooney and Miller 1982).

Interrelationships Between Grain Characteristics of Colored-Grain Sorghum

Significant correlations of a low magnitude were observed between many of the grain characteristics (Table II). A positive

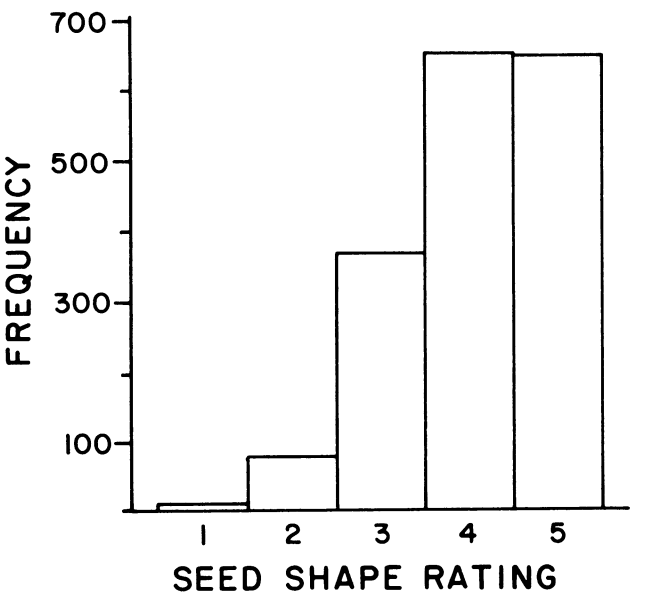

Fig. 4. Seed shape (1 = round, 5 = flat) distribution among colored-grain sorghum lines (n = 1,768). The minimum, mean, and maximum seed shape ratings were 1.0, 4.1, and 5.0, respectively.

correlation ($r = 0.54$, $P < 0.01$) was observed between tannin content and the blank reading for the total population. Weaker relationships existed between tannin content and blank reading for the high-tannin ($r = 0.43$, $P < 0.01$) and the low-tannin ($r = 0.43$, $P < 0.01$) groups. The blank reading represents the concentration of the methanol-soluble phenolic compounds present in the grain. Considering these low correlation coefficients, it is unlikely that the blank reading could be used successfully to predict tannin content.

Tannin content was negatively correlated with AHI for the total population ($r = -0.37$, $P < 0.01$) and for the high- ($r = -0.24$, $P < 0.01$) and low-tannin ($r = -0.13$, $P < 0.05$) groups. However, tannin content accounted for only a small proportion (coefficient of determination = 1.7–18.5%) of the observed variability in AHI in all groups.

When the total population was considered, tannin content was negatively correlated ($r = -0.43$, $P < 0.01$) with seed weight. The correlation coefficient was slightly enhanced ($r = -0.46$, $P < 0.01$) for the high-tannin group and not significant ($r = 0.04$, $P > 0.05$) for the low-tannin group. The sorghum line IS 9215, which recorded the highest tannin level (11.8%), also had the smallest seed weight (1.0 g/100 seeds).

Seed color and tannin content were positively correlated ($r = 0.36$, $P < 0.01$) for the total population. A similar relationship was evident for both the high- ($r = 0.16$, $P < 0.01$) and the low-tannin ($r = 0.42$, $P < 0.01$) groups. The correlations between seed color and

TABLE I
Color Characteristics, Tannin Content, and Abrasive Hardness Index of Color Classes of Colored-Grain Sorghum Lines (n = 1,768)

Color Class[b]	Visual Description	Number of Samples	Grain Color[a]			Range in Tannin Content (%)	Range in Abrasive Hardness Index (sec)
			L	*a*	*b*		
1	Light gray	88	53.2	1.0	9.2	0.0–3.4	3.8–19.8
2	White with brown spots	12	51.7	4.7	15.9	1.7–4.4	3.7–10.3
3	Purple with white spots	8	47.8	3.7	11.2	0.0–0.6	4.7–14.7
4	Light red with white spots	48	46.8	6.7	14.4	0.0–5.5	4.3–21.3
5	Gray yellow	56	45.7	2.1	13.3	0.0–5.2	3.4–19.6
6	Light red	24	45.2	9.0	16.7	0.1–6.9	3.4–18.3
7	Light brown	57	44.4	5.7	16.9	0.0–7.5	3.5–13.2
8	Reddish gray	23	43.9	4.3	11.7	0.0–6.9	3.1–15.1
9	Light brown yellow	174	40.0	7.2	16.6	0.4–8.2	3.0–15.1
10	Light brown yellow	203	38.7	8.8	15.9	0.0–9.7	3.0–17.2
11	Reddish yellow	14	37.2	9.7	14.7	0.2–7.4	3.5–12.0
12	Red	72	34.7	15.6	15.5	0.0–5.9	3.2–17.7
13	Light brown	71	33.1	8.5	13.7	0.2–7.9	3.0–17.6
14	Brown yellow	7	32.9	6.8	12.3	2.1–4.8	4.1–11.7
15	Brown	171	32.6	12.2	13.5	0.0–9.0	2.5–17.2
16	Brown	108	31.9	10.9	13.5	0.0–7.8	3.1–14.8
17	Brown	48	31.7	9.2	11.3	0.7–11.8	3.0–12.2
18	Purple brown	28	31.2	9.6	10.2	0.1–5.7	3.3–16.3
19	Reddish brown	30	30.6	13.8	11.9	0.3–11.1	3.1–12.0
20	Dark yellow brown	77	27.0	7.9	10.6	0.1–7.2	3.2–19.6
21	Dark red	237	26.3	11.8	9.5	0.1–10.2	2.6–18.8
22	Dark brown	62	23.8	12.1	7.8	1.0–10.3	27.–12.4
23	Dark brown	146	22.3	10.1	6.2	0.0–9.9	2.5–14.2
24	Dark brown	10	21.3	8.5	5.1	3.0–8.5	3.0–11.6

[a] Measured with a Hunterlab color difference meter in comparison to a standard disk ($L = 94.7$, $a = -0.9$, and $b = 0.5$).
[b] Ranked in order of decreasing L value (white = 100, black = 0).

TABLE II
Linear Correlation Coefficients Between Grain Characteristics of Colored-Grain Sorghum Lines

Sorghum Lines	Blank Value	Abrasive Hardness Index	Seed Weight	Seed Shape	Seed Color
Total population (n = 1,768)					
Tannin content	0.54**[a]	−0.37**	−0.43**	0.31**	0.36**
Blank value[b]		−0.33**	−0.28**	0.12**	0.57**
Abrasive hardness index			0.06*	−0.10**	−0.23**
Seed weight				−0.23**	−0.20**
Seed shape					...
Lines with tannin > 1% (n = 1,421)					
Tannin content	0.43**	−0.24**	−0.46**	0.21**	0.16**
Blank value[b]		−0.26**	−0.26**	0.01	0.50**
Abrasive hardness index			0.05	−0.05	−0.12**
Seed weight				−0.21**	−0.16**
Seed shape					...
Lines with tannin < 1% (n = 347)					
Tannin content	0.43**	−0.13*	0.04	0.06	0.42**
Blank value[b]		−0.06	0.13*	0.03	0.49**
Abrasive hardness index			−0.19**	0.11	−0.04
Seed weight				0.08	−0.16**
Seed shape					...

[a] ** $P < 0.01$, * $P < 0.05$.
[b] Determined in the vanillin-HCl assay for tannin content.

tannin content, although significant, were weak, suggesting that use of seed color as an indicator of tannin content could be misleading, which is in agreement with conclusions made by Bullard et al (1981) and Subramaniam et al (1983).

Identification of High-Tannin Lines with Good Dehulling Characteristics

Figure 5 shows the percentage of the total population of colored-grain sorghum lines that met the various selection criteria used to identify high-tannin lines with good dehulling characteristics. Of the 1,768 lines, 90 had a tannin content greater than 1% and an AHI greater than 11.5 sec. These represent the high-tannin lines with at least 50% corneous endosperm (Mwasaru 1985). A subset of 45 of these lines was chosen for a dehulling test, and of these, 28 could be dehulled with a flour extraction (tannin) of at least 70%, which would be acceptable under commercial conditions. For these 28 lines, the flour extraction (tannin) was highly correlated ($r = -0.89$, $P < 0.01$) with the tannin content indicating that within hard, high-tannin lines, higher flour extraction levels could be obtained with lines lower in tannin.

Only 10 of a subset of 15 of the 28 lines could be dehulled in the TADD with a flour extraction of at least 70% to yield flour with a color reading of 30% measured on an Agtron reflectance spectrophotometer (Table III). Only four of the 10 lines (IS 8186, 9700, 6902, and 21054) gave a color reading of 35% with a flour extraction of 70% or higher; the color of these flours would be considered acceptable, at least in some regions of Nigeria (Reichert and Youngs 1976). Only two of six lines that were identified as having an AHI greater than the mean of 21 noncolored lines had good dehulling quality; this suggests that although hardness is an important criterion for good dehulling quality, other factors must also be important. Line 8186 had the highest flour extraction levels, perhaps because the grain had the roundest shape of any of the lines in Table III and because it was also harder, smaller, and lower in tannin than eight of the other lines. The lines with the flattest grain (IS 7994, 9689, and 9185) generally showed the lowest flour extraction levels.

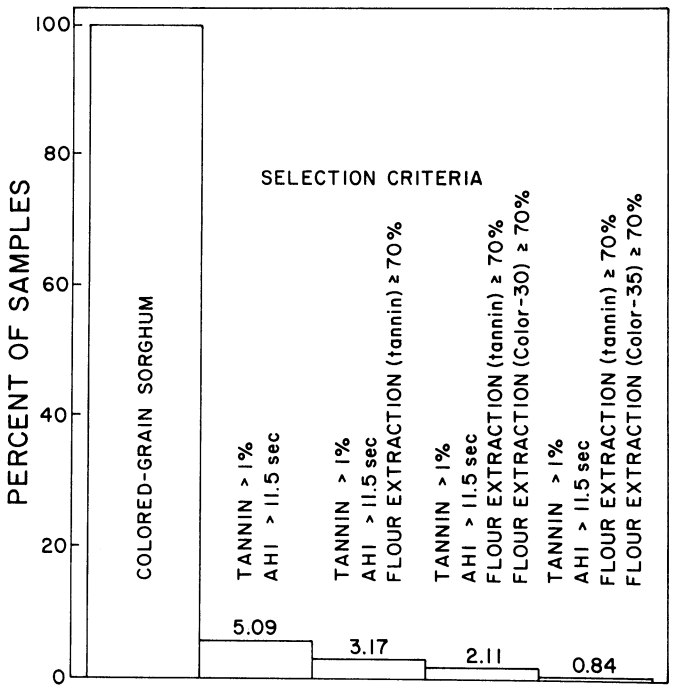

Fig. 5. Proportions of the colored-grain sorghum population ($n = 1,768$) identified on the basis of the criteria adopted for selecting lines with good milling characteristics.

SUMMARY AND CONCLUSIONS

Eighty percent of the colored-grain sorghum lines analyzed contained greater than 1% tannin. Most of the colored-grain lines (85%) had tannin contents of 5% or less. Five lines with greater than 10% tannin were identified; these lines or the bran fractions obtained from them, in particular, could serve as raw material for industrial-scale production of sorghum tannins, if these tannins had similar properties to those which are commercially available. High-tannin lines were slightly softer than the low-tannin colored-grain lines and much softer than noncolored sorghum lines; only 0.3% of colored-grain lines exceeded the mean hardness of 21 noncolored lines. Although the texture of colored-grain lines as a group was softer than noncolored lines, there was only a low but significant correlation ($r = 0.37$, $P < 0.01$) between tannin content of colored-grain lines and grain hardness. Most lines had flat seeds, and 70% had seed sizes of 3.0 g/100 seeds or less. The colored-grain lines exhibited a diversity of color ranging from light gray to dark brown, and 24 color classes could be visually distinguished. With the exception of one color class, the lines within each class varied very widely in tannin content and AHI. Several sorghum lines in three color classes lacked brown or reddish pigmentation associated with the presence of the spreader gene (S), yet contained tannin contents greater than 1%. Since low but significant correlations were observed between grain characteristics (tannin content, AHI, seed shape and color), it was concluded that use of one of these grain characteristics to predict another could result in serious errors.

Four high-tannin lines (>1%) were identified that could be

TABLE III
Characteristics of the High-Tannin Sorghum Lines with Good Dehulling Quality

Line (IS Number)	Tannin (%)	Abrasive Hardness Index (sec)	Seed Weight (g/100 seeds)	Seed Shape[a]	Color Class[b]	Flour Extraction (Tannin)[c] (%)	Flour Extraction (Color-30)[d] (%)	Flour Extraction (Color-35)[e] (%)
8186	1.3	19.6	1.9	3	9	89	81	74
9700	1.2	12.1	3.5	4	17	88	78	72
6902	1.7	12.0	3.2	4	6	87	80	70
21054	3.4	19.6	1.9	4	5	72	75	70
2832	2.1	11.6	2.6	4	17	84	80	65
8145	2.7	14.1	2.9	4	14	73	75	62
1337	2.4	13.0	2.1	4	5	72	79	60
7994	2.1	12.0	2.5	5	12	77	71	55
9689	1.6	12.1	2.8	5	12	82	73	54
9185	1.5	15.3	2.0	5	9	85	73	52
Range	1.2–3.4	11.6–19.6	1.9–3.5	3–5	5–17	72–89	71–81	52–74

[a] The shape rating is on a scale of 1 (spherical seed) to 5 (flat seed).
[b] Refer to Table I for the description of each color class.
[c] Extraction level required to produce flour containing 0.5% tannin content.
[d] Extraction level required to produce flour with a color reading = 30% measured on an Agtron reflectance spectrophotometer.
[e] Extraction level required to produce flour with a color reading = 35% measured on an Agtron reflectance spectrophotometer.

dehulled, in the laboratory, with extraction levels of at least 70% to yield flour that contained less than 0.5% tannin and that was equivalent in color to flour considered acceptable for consumption. These lines need to be tested in multilocation trials and in commercial-scale dehulling equipment to confirm their superior milling performance.

ACKNOWLEDGMENTS

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