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Some Agronomic and Biochemical Characters of Brown Sorghums and Their Possible Role in Bird Resistance

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Chemical composition including tannin content and grain and agronomic characters including earhead length, head type, glume color, and threshability are reported for 18 sorghum genotypes with brown pericarp color, 15 of which had been reported to be bird resistant. Agronomic characters varied significantly among the genotypes. Variation in tannin content was much larger than variation in the other constituents. Detailed polyphenol analysis on selected genotypes indicated that some lines had insignificant levels of condensed tannins, that none of them was a group II sorghum, and that the levels of flavan-4-ols were relatively high. The possible role of polyphenolic components in relation to bird resistance is discussed.

Sorghum is a major staple food grain crop on the African and Asian continents. One of the major constraints on the production of grain sorghum is the severe bird depredation in many areas of Africa and many developing countries (Bullard and Elias, 1980). Sorghum produced in these areas is usually limited to bird-resistant cultivars, which are generally found to contain relatively high concentrations of polyphenols such as the condensed tannins (Tipton et al., 1970; Hoshino and Duncan, 1980). Brown-seeded hybrids have been reported to contain higher tannin levels than red- or yellow-seeded hybrids (Harris, 1969), and seed color of sorghum showed a highly significant positive correlation with tannin content (McMillan et al., 1972). However, Mabbayard and Tipton (1975) reported that pericarp color may not be a reliable indicator of tannin concentration.

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The astringency of the tannins is considered to be the principal means by which high-tannin sorghums effect resistance to bird depredation (Bullard and Elias, 1980). Resistance to bird depredation is a complex phenomenon that may be associated with non-tannin polyphenols, as well as tannins (Bullard et al., 1980). Flavan-4-ol monomers may contribute to bird repellency of high-tannin sorghums (Butler, 1982). Tannins in sorghum have also been associated with reduced preharvest germination and grain molding when wet weather prevails at the time of harvest (Harris and Burns, 1970). Besides the above advantages, high-tannin sorghums tend to be less digestible and nutritionally inferior to sorghums in which tannin is absent or is present at low levels (Maxson et al., 1973; Featherston and Rogler, 1975; Jambunathan and Mertz, 1973). However, tannin-containing varieties classified as group II may be nutritionally similar to non-tannin varieties (Oswalt, 1975).

The present report describes the variation in agronomic characters and chemical constituents of sorghum grains from 18 different genotypes. Fifteen genotypes selected in the study have been reported to be "bird-proof" sorghums (AICSIP, 1971) and their grains had brown peri-

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Table I. Agronomic Characters of Brown Sorghum Genotypes

IS no	origin	earhead length, cm	head type[a]	glume color[b]	glume covering[c]	corneousness[d]	threshability
2849	S Africa	25 5	CE	BL	3	4	PT
3035	S. Africa	27 0	SLSB	S	4	4	FT
8765	S. Africa	21.0	SCE	BL	3	3	PT
3149	S Africa	20.5	CO	BL	3	3	PT
3153	S Africa	27.5	SCO	S	3	5	PT
962	U S A	26.0	SLSB	LB	3	3	PT
2827	Zimbabwe	23 5	CO	S	3	5	FT
10301	Indonesia	28.0	SLSB	PSP	2	4	PT
1109	India	13 5	SLSB	P	2	4	FT
8748	S Africa	30 0	SCO	BR	2	5	PT
8746	S Africa	15 5	SLSB	P	2	4	FT
3150	S Africa	20 5	LSB	BL	5	3	DT
2826	Zimbabwe	22 5	CO	BR	3	5	FT
3171	S. Africa	29 5	CO	P	3	5	PT
8754	S Africa	25 0	SLSB	BL	5	3	DT
724	U S A	17 0	CE	P	3	4	PT
2880	Greece	13 5	CO	LR	3	5	PT
3031	Ethiopia	18 0	CE	R	2	4	FT

[a] Head type CE = compact elliptic, SLSB = semiloose with stiff branches, CO = compact oval, LSB = loose with stiff branches, SCO = semicompact oval, SCE = semicompact elliptic [b] Glume color P = purple, LB = light brown, BR = brown, S = straw, BL = black, LR = light red, R = red, PSB = partly straw and purple [c] Glume covering 2 = 0 25 grain covered, 3 = 0 50 grain covered, 4 = 0 75 grain covered, 5 = grain fully covered [d] Corneousness of grain 3 = partly corneous, 4 = almost floury, 5 = completely floury [e] Threshability FT = freely threshable, PT = partly threshable, DT = difficult to thresh

carps. In order to be consistent, we have referred to these "bird-proof" sorghums as bird-resistant sorghums in this paper. Three additional genotypes, IS-724, IS-2880, and IS-3031, were selected from our germ plasm accessions because they also had brown pericarps. These 18 genotypes were originally collected from Ethiopia, Greece, India, Indonesia, South Africa (S. Africa), United States of America (U.S.A.), and Zimbabwe. Since plant characteristics such as loose and pendant heads, large glumes, and awns have been reported to be associated with reduced bird damage of sorghum grains (Bruggers and Jaeger, 1982), the 18 genotypes were evaluated for appropriate agronomic characters. We have subjected the grain samples to a variety of chemical analyses, with particular attention to the polyphenol components (including flavanols), since polyphenols have been reported to be associated with bird resistance (Bullard et al., 1980).

EXPERIMENTAL SECTION

Sorghum grain was obtained from the post-rainy-season harvest of 1978 at ICRISAT Center, Patancheru, India, and the origin of these sorghum cultivars is shown in Table I. The description of the 18 lines selected for this study is given in AICSIP (1971).

Plant and Grain Characters. The agronomic characters of the genotypes such as the earhead length, head type, and glume characters were recorded at ICRISAT. Corneousness of grain was visually scored after cutting 10 grains and observing the proportion of floury/corneous endosperm. A scale of 1–5 was used where 1 = <10% and 5 = >90% floury endosperm. The color of grains was judged by using the Munsell color chart (Munsell Color Co., Maryland). The breaking strength of the grain (in kg) was determined by using the Kiya hardness tester (Kiya Seisakusho Ltd., Japan). The mean values were calculated from measurements of 20 individual grains.

Chemical Analyses. Analyses reported in Table II were conducted at ICRISAT. The grain samples were dried at 37 °C for 48 h and ground in a Udy cyclone mill (UD Corporation, Boulder, CO) to pass through a 0.4-mm screen. The fat content was estimated by extracting the flour with *n*-hexane for 5 h with a Soxhlet apparatus (AOAC, 1975). The chemical analyses were duplicated from the defatted flour for each grain sample and the mean values are given. Standard error (SE) of estimation for each of the chemical constituents was determined by analyzing one sample at least 10 times. The tannin content was determined as presented in Table II by the vanillin assay technique (Burns, 1971); the values were calculated by substracting the blank (Price and Butler, 1977) with catechin as the standard. The results are reported as catechin equivalents (CE). Crude protein ($N \times 6.25$) was determined by using the micro-Kjeldahl procedure (AOAC, 1975). Starch was determined by using the enzyme glucoamylase (Sigma) as reported by Singh et al. (1980). For estimation of soluble sugars, the flour was extracted with 80% ethanol for 6 h in a Soxhlet apparatus. After evaporating the ethanol extract in vacuo, the contents were dissolved in water. Total sugars were determined by the phenol–sulfuric acid method (Dubois et al., 1956).

Polyphenol analyses presented in Table III were carried out at Purdue University. Single 5-g samples of grain from which all glumes, broken grains and debris had been removed were ground for 2 min in an analytical mill (Model A10, Tekmar Co., Cincinnati, OH). Four hundred milligrams of flour was extracted twice with 10 mL of methanol for 15 min at room temperature with continuous agitation. The methanol extracts were combined for analyses, and the residue, which was separated from the extracts by centrifugation, was similarly reextracted twice with methanol containing 1% (v/v) concentrated HCl. Polyphenol analyses were carried out on both methanol and acidic methanol extracts within 36 h of extraction.

Total phenols were estimated by the Prussian blue method of Price and Butler (1977) standardized with $FeSO_4$. Assays of protein precipitation capacity were carried out as described by Hagerman and Butler (1980) using bovine serum albumin labeled with ^{14}C according to the method of Jentoft and Dearborn (1979). The vanillin assay for flavan-3-ols and their oligomers was carried out as described by Price et al. (1978), using catechin as a standard. Determination of flavan-4-ols and proanthocyanidins by their conversion to anthocyanidins in 30% (v/v) concentrated HCl in 1-butanol was carried out as described by Watterson and Butler (1983), with the exception that total extracts as well as only the components

Table II. Grain Characters and Chemical Composition of Brown Sorghum Genotypes[d]

IS no.	description[a]	color of grain	Munsell color coding	corneousness[b]	100 grain wt, g	breaking strength, kg[c]	tannin, CE	% protein	% fat	% starch	% soluble sugars
2849	bird-proof H. Potchestroom DL/59/1526	light yellowish red (red and yellow mixed)	2.5 YR 4.5/6	4	3.44	3.0	0.13	10.6	2.9	73.1	1.4
3035	K_2 sorghum bird proof	brick red with yellow	2.5 YR 4/6	4	2.02	5.2	0.14	9.0	2.8	74.9	1.4
8765	E 256 bird proof DL/60/84	light reddish brown	2.5 YR 4/6	3	2.68	5.7	0.23	11.1	3.0	71.5	1.3
3149	DL/60/103 bird proof	brick red with yellow	2.5 YR 4.5/5	3	2.29	5.4	0.24	10.0	3.1	73.4	1.3
3153	DL/60/110 bird proof (old type)	light reddish brown	2.5 YR 4/6	5	1.92	2.8	0.27	9.2	2.7	73.7	1.5
962	bird and striga resistant (PI 239440)	light (brick) reddish brown	2.5 YR 4/6	3	2.22	3.2	0.37	8.8	2.8	74.9	1.4
2827	bird proof 224/56 ex. U. of Africa (South)	light reddish brown	2.5 YR 4/6	5	1.80	2.3	0.43	10.0	3.2	73.2	1.5
10301	bird proof (Thailand)	dark brown	2.5 YR 2.5/6	4	2.74	4.2	0.49	9.8	2.9	71.6	1.4
1109	Ladore (BR) SA 67/2	reddish brown	10 R 3.5/6	4	1.91	4.1	0.62	13.2	3.5	69.1	1.5
8748	E 238 bird proof DL/60/64	dark brown	2.5 YR 2.5/6	5	2.42	2.7	0.86	8.9	3.4	70.6	1.6
8746	E 236 bird proof (old type) DL/60/62	light yellow with brown tinge		4	2.90	8.4	1.00	11.1	2.7	67.2	1.6
3150	DL/60/105 bird proof (improved)	dull brown	5 YR 3.5/6	3	1.67	2.4	1.34	11.9		71.6	1.4
2826	bird proof 196/51	dull brown	2.5 YR 3.5/4.5	5	1.82	3.6	2.10	10.7	2.7	71.2	1.6
3171	DL/60/136 bird proof	dark brown	2.5 YR 2.5/6	5	2.26	3.0	2.14	10.0	3.1	69.6	1.9
8754	E 244 bird proof improved DL/60/70	dull brown	2.5 YR 3/6	3	1.29	3.7	2.18	12.8	4.9	69.5	1.1
724	Sumac 6550	dark brown	2.5 YR 2.5/6	4	1.33	3.3	3.68	10.4	3.5	68.0	2.0
2880	FAO No. 8748 M-6509 local variety Ipicos	dark brown	2.5 YR 2.5/6	5	1.48	2.0	5.72	11.7	2.4	66.2	2.5
3031	No. 125 from Dunbidola	dark brown	2.5 YR 2.5/6	4	1.76	6.8	7.22	10.9	2.2	66.9	2.4

[a] AICSIP (1971). We have retained the words "bird proof" as it appeared in the original reference. [b] Corneousness (given in Table I also for comparison): 3 = partly corneous; 4 = almost floury; 5 = completely floury. [c] Measured as force in kg to break the grain using a Kiya hardness tester. [d] Standard error of estimation (SE) for tannin (CE), 0.064, protein, 0.10, fat, 0.06, starch, 2.36, and soluble sugars, 0.03.

Table III. Polyphenol Analyses

IS no.	extractant	total phenols, μmol/g of seed	protein precipitation, mg of protein/g of seed	vanillin assay, g of catechin/100 g of seeds	proantho-cyanidins, A_{500}/g	flavan-4-ols, A_{500}/g
2849	methanol	64	3.28	0.01	0	2.9
	H⁺/methanol	22	3.83	0	0	2.1
8765	methanol	78	0.37	0.02	1.3	19.7
	H⁺/methanol	27	0.09	0	0.6	4.1
3153	methanol	61	1.94	0.02	1.3	17.3
	H⁺/methanol	45	0	0	0.6	4.3
2827	methanol	55	0	0.02	1.6	17.1
	H⁺/methanol	35	2.15	0.01	0.7	5.8
8748	methanol	149	0	0.20	9.2	13.4
	H⁺/methanol	116	0	0.13	9.2	4.7
2826	methanol	254	28.30	0.76	26.1	15.1
	H⁺/methanol	116	1.92	0 13	10.9	3.5
3171	methanol	297	20.80	0.63	23.4	17.3
	H⁺/methanol	137	0.88	0.19	11.2	3.5
724	methanol	419	47.50	1.14	29.0	22.1
	H⁺/methanol	186	4.64	0.26	13.7	4.3
2880	methanol	514	60.50	1.89	41.7	20.8
	H⁺/methanol	265	8.17	0.46	20.9	5.1
3031	methanol	714	74.30	2.43	46.4	32.9
	H⁺/methanol	394	17.20	0.74	27.2	7.9

that are bound by insoluble polyvinylpyrrolidone (PVP) were measured. PVP-bound materials gave values that were usually 60–70% as large as the total in the extract, and these values are presented in Table III.

RESULTS AND DISCUSSION

The agronomic characters were studied to determine the variability among the cultivars. The 18 genotypes showed considerable variation in their argonomic characters, namely, earhead length, head type, glume color, glume covering, corneousness, and threshability (Table I). Since the agronomic characters differ significantly, they are not likely to be responsible for bird resistance. However, it should be emphasized that these cultivars should be tested for their bird-resistant characteristics under actual field situations.

The grain characters of the 18 genotypes are given in Table II. The grains of these genotypes are generally small and their 100-grain weight ranged between 1.3 and 3.4 g. Breaking strength of grains varied from 2.0 to 8.4 kg as measured by a Kiya hardness tester. The grains of IS-3031 and IS-8746 showed higher values for breaking strength. Such grains may withstand breakage during the dehulling process for the removal of tannin, thus improving the milling yield and utilization of the grain. The degree of grain corneousness ranged from partly corneous to completely floury. Brown sorghum grains from East Africa usually have floury endosperm and possess insufficient strength to withstand polishing (Shepherd, 1974).

The fat and protein contents varied from 2.2 to 4.9 and 8.8 to 13.2%, respectively, among the 18 genotypes (Table II). The sugar and starch contents did not vary appreciably. The data show that these grains possess satisfactory amounts of the chemical constituents that are nutritionally important.

The tannin content (catechin equivalents) ranged from 0.13 to 7.22, though all these lines had a brown pericarp (Table II). Although the grains of IS-10301, -8748, -3171, -724, -2880, and -3031 possessed identical color (Munsell color coding 2.5 YR 2.5/6), they had 0.49, 0.86, 2.14, 3.68, 5.72, and 7.22 CE values, respectively. Similarly, the CE values differed for IS-3150 and -8754, though the grains had similar pericarp color. The data indicate that pericarp color may not be an indicator of tannin concentration. Although all the genotypes except IS-724, -2880, and -3031 were reported to be bird resistant, several of them contained only small quantities of tannin according to the vanillin assay. Mabbayard and Tipton (1975) reported that it may be possible to maintain a satisfactory degree of bird resistance at lower tannin levels since certain types like DeKalb × 1602, McCurdy 11, and ACCO × 9417BR were low in tannin but exhibited a high degree of bird resistance.

Bird resistance is usually associated with relatively high levels of tannin (Tipton et al., 1970). Because some of these lines had low tannin levels as determined by the vanillin assay (Table II), 10 of the cultivars (including IS-724, -2880, and -3031 with high condensed tannins values but which were not reported as bird-resistant types) were subjected to a series of assays for various polyphenol components (Table III). None of these 10 samples appeared to be a group II sorghum, with polyphenols extractable only in acidic methanol (Price and Butler, 1977). Assays that measure condensed tannins (protein precipitation, vanillin and proanthocyanidin) generally agreed that grains of IS-2826, -3171, -724, -2880, and -3031 contained condensed tannins in amounts that might be expected to confer bird resistance (Bullard et al., 1980). The other lines did not contain significant amounts of condensed tannins. The nutritional value of these low-tannin lines (IS-2849, -8765, -3153, -2827, and -8748) may be considerably greater than high-tannin lines (IS-3031 or IS-2880) because high-tannin lines are poorly digestible (Harris et al., 1970).

The flavan-4-ols are readily converted to anthocyanidins in acidic solvents at room temperature and have therefore been designated as leucoanthocyanidins, to distinguish them from the polymeric proanthocyanidins (condensed tannins) that yield anthocyanidins on heating in strong acids (Watterson and Butler, 1983). In a survey of 43 sorghum cultivars, flavan-4-ols were detected in the seeds of only 15 lines (Watterson and Butler, 1983) and the amount found in most of these genotypes was low, comparable to the level for IS-2849 reported in Table III. The amounts of flavan-4-ols reported in Table III for the other nine varieties were considerably higher than those found in the previous survey. The average value for the 9 varieties (excluding IS-2849) in Table III is 19.5 A_{550}/g (ab-

sorbance at 550 nm for the extract in 1 g of flour) whereas the highest amount found in the 43 varieties not selected for their bird resistance was 16 A_{550}/g. The incidence of lines relatively rich in flavan-4-ols in this population of sorghum suggests that the presence of flavan-4-ols in the seed may be a factor in the reported bird-resistant properties (AICSIP, 1971). In future studies of bird resistance, attempts will be made to establish relationships between bird resistance and flavan-4-ols, condensed tannins, other polyphenols, or other seed characteristics. Lines with high quantities of condensed tannins (IS-724, -2880, and -3031) should be checked for bird resistance/susceptibility. A better understanding of the nature and chemistry of polyphenols including tannins may be fruitful in elucidating the factors responsible for bird resistance in sorghum. It has been reported that some cultivars have a high level of tannin (likely to be bird resistant) in the immature stages when bird damage is expected to be most serious and a low tannin level (and presumably high nutritional quality) in the mature grain (Butler et al., 1980). It may be possible to strike an optimal balance for both these qualities through genetic selection.

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