Effect of Genotype on Tannins and Phenols of Sorghum[1]

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ABSTRACT

The color and the phenols of sorghum pericarp and testa are controlled by the *R*, *Y*, B_1, B_2 and *S* genes. Relatively pure fractions of pericarp, testa, and endosperm were isolated by abrasive milling, sieving, and air flotation from six genetically diverse sorghum varieties grown at two locations. Ground whole grain, pericarp, testa, and endosperm were extracted sequentially with methanol, acidic methanol (1% HCl), and dimethylformamide. The phenolic compounds were measured and characterized by four methods: vanillin assay, Folin-Ciocalteu assay, anthocyanidin pigment determination, and relative degree of polymerization (RDP). The endosperm did not contain significant amounts of extractable phenols. *R* and *Y* genes did not affect the level of Folin-Ciocalteu positive phenols or tannins in group I sorghums ($b_1b_2B_1$-, B_1-b_2b_2, $b_1b_1b_2b_2$). Dominant *R* and *Y* greatly increased the levels of anthocyanidin pigments in group I ($b_1b_1B_2$-) and II (B_1-B_2-*ss*) sorghums. Group II sorghums with a red pericarp (*R*-*Y*-B_1-B_2-*ss*) had higher levels of Folin-Ciocalteu-positive phenols or tannins in group I sorghums ($b_1b_2B_1$-, B_1-b_2b_2, with a white pericarp (*R*-*yy*-B_1-B_2-*ss*). The RDP of the tannins in the testa was not affected by *R* or *Y*, but the tannins in the pericarp of group II sorghums with a white pericarp had a higher RDP. The level of tannins in the testa of group III sorghums was not affected by *R* and *Y*, but more Folin-Ciocalteu phenols were extracted with methanol from the testa when the pericarp was white (*R*-*yy*-B_1-B_2-*S*). The pericarp and testa of group III sorghums (B_1-B_2-*S*-) had similar levels of anthocyanidin pigments. Group III sorghums had higher levels of Folin-Ciocalteu phenols and tannins in the whole grain, pericarp, and testa fractions than group II sorghums. The *S* gene did not affect the RDP of the tannins but did affect the extractability.

Sorghum (*Sorghum bicolor* (L.) Moench) is the fifth leading cereal crop in total world production behind rice, wheat, corn, and barley and is typically grown under hot, dry conditions. The color of sorghum grain varies greatly and is caused by several interacting factors, including pericarp color and thickness, presence of a testa, and endosperm texture and color. Pigmentation of the pericarp and testa is due to phenolic compounds.

The *R*, *Y*, B_1, B_2, and *S* genes are known to control the color (Rooney and Miller 1982) and pigmentation of the pericarp (Nip and Burns 1969, 1971). There are pigments or pigment precursors present in the epicarp of nearly all sorghums, regardless of color. The amount and color of these pigments changes with the *R*, *Y*, B_1, B_2, and *S* genes. The *R* and *Y* genes determine the color of the pericarp and the B_1 and B_2 genes control the presence of a pigmented testa. Sorghums with a pigmented testa contain high levels of condensed tannins. Phenols, especially tannins, protect the seed from attack by molds, insects, and birds and from preharvest germination (Hahn et al 1983, Butler 1982a). Tannins provide the greatest amount of protection, but, for most livestock species, diets containing high-tannin sorghum are significantly less digestible than those containing sorghum without tannins (Hahn et al 1984).

Sorghum varieties have been subdivided into three groups according to the levels of phenols present, primarily tannins (Cummings and Axtell 1973, Butler and Price 1977), and the genotype of the grain (Rooney and Miller 1982). Group I sorghums do not have a pigmented testa, contain low levels of phenols and no tannins. Group II and III sorghums both have a pigmented testa. The tannins of group II sorghums are extracted in significant amounts by acidic methanol (1% HCl) but not by methanol alone. In group II sorghums, the spreader (*S*) gene is recessive. *S* is dominant in group III sorghums, and their tannins can be extracted in significant amounts by both methanol and acidic methanol. Specific effects of the *S*, *R*, *Y*, and *I* genes on the tannins and phenols of sorghum are not known.

Shepherd (1981) found that during decortication the pericarp separated from the rest of the kernel at the cross and tube cells. Thus, it is feasible to remove "pure" fractions of pericarp, testa, and endosperm from sorghum kernels using the abrasive mill of Shepherd (1981) or another type (Oomah et al 1981).

The purpose of this study was to determine the effects of genotype and growing location on the sorghum kernel and the tannins and phenols of sorghum. First, fractions of pericarp, testa, and endosperm were isolated. Then the phenols and tannins were characterized by chemical methods.

MATERIALS AND METHODS

Samples

Sorghum varieties differing in *R*, *Y*, B_1, B_2, and *S* genes were grown at Halfway and College Station, Texas, in 1981 and 1983, respectively (Table I). The grain was harvested, cleaned, and stored at −4° C until used.

Isolation of Pericarp and Testa Fractions

Relatively pure fractions of pericarp and testa were isolated by abrasive milling, air flotation, and sieving. One kilogram of clean, sound grain was milled using an abrasive mill equipped with seven resinoid disks (Oomah et al 1981) until most of the pericarp was removed (75–100 sec). The pericarp was removed in flakes (Shepherd 1981) and separated by air flotation. The intact pearled kernels (without pericarp) were milled a second time (30–45 sec) and the fines collected. Preliminary studies (using ATx623 × SC0103) revealed that the throughs of a U.S. #40 and the overs of a U.S. #60 mesh sieve contained the highest levels of tannins. This fraction was called the "testa"and appeared to be a concentration of the testa layer, containing some endosperm and very small amounts of pericarp tissue. The milling fractions were stored in sealed containers at −4° C until they were analyzed.

Compositional Analysis

Moisture content of the samples was determined by AACC method 40-01 (1976). Crude protein (N × 6.25) was determined by the Kjeldahl method using a Technicon AutoAnalyzer IIC system (Technicon 1977). For starch determination, a ground sample was autoclaved in 20 ml of distilled water at 120° C for 2.5 hr, then incubated with 25 ml of acetate buffer (pH 4.5) and 1 ml of glucoamylase (Diazyme L-100, Miles Laboratory, Inc., Elkhart, IN). The amount of glucose released was measured using a Technicon AutoAnalyzer IIC system (Technicon 1978).

Extraction

Samples of whole grain, pericarp, "testa," and endosperm were ground in a Udy laboratory mill to pass through a 0.1-mm screen. Ground samples (0.1–0.2 g) were extracted for 2 hr with 8 ml of methanol (McDonough et al 1983). After centrifugation, the solvent was removed and the residue extracted for 2 hr with acidic methanol (1% HCl) to remove phenolic compounds not extracted by methanol, and a third time for 2 hr with dimethylformamide (DMF) to remove any phenolic compounds remaining.

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Assays

Total phenolic content of each extract was measured by the automated Folin-Ciocalteu method of Kaluza et al (1980). Tannin content of the extracts was measured by the vanillin hydrochloric acid procedure with blanks (Price et al 1978). The 2-hr extraction was longer than the 20 min normally used for the vanillin assay. Absorption of the extract (1% HCl in methanol) in the vanillin assay may decrease slightly after 20 min (Price et al 1978), but this should not affect the relative level of tannins measured in the acidic methanol extract. The blanks of the vanillin assay were used as a measure of anthocyanidin pigments in the extracts. This assay measured the red pigments in the extracts, including both anthocyanidin and anthocyanin pigments. Anthocyanins are glucosides of anthocyanidins and are readily converted to their corresponding anthocyanidins in acid medium. Relative degree of polymerization (RDP) of the tannins was determined as described by Butler (1982b). The RDP was calculated as the ratio of absorbance of flavan-3-ol monomer units to the absorbance of the terminal flavan-3-ol residues. Total flavan-3-ol monomers were measured by their red color formation in acidic butanol (30% HCl). Terminal flavan-3-ol residues (tannin oligomers) were measured by the vanillin assay as modified by Butler et al (1982), who replaced methanol with glacial acetic acid in the vanillin reagent.

Pronase Inhibition

Inhibition of pronase hydrolysis was determined by a modification of the protein availability assay of Hahn et al (1982). The enzyme concentration was reduced from 2 mg/ml pronase (0.2*M* citrate-phosphate buffer, pH 7.0) to 0.5 mg/ml pronase. Approximately 25 mg of protein was weighed into each tube and 20 ml of enzyme solution added. Between 70 and 75% of the protein was added as casein, and the remainder was added from the milling fractions. Casein was used as a 100% digestible control.

RESULTS AND DISCUSSION

Preparation of Milling Fractions

Varieties with thick starchy mesocarps were chosen where possible to facilitate the isolation of pericarp tissue (Table II). Viewed with low-magnification microscopy, the pericarp fractions of all the varieties were flakes with smooth, white, concave surfaces on the undersides resulting from the presence of the starchy mesocarp. The varieties Tx2566 and ATx399 × RTx430 had thin pericarps and therefore produced the smallest flakes. In addition, these two varieties had the highest levels of protein in the pericarp. This does not, however, appear to be caused by endosperm contamination, as none was visible. Starch has been reported in the pericarp of sorghum (Earp and Rooney 1982) and would account for the relatively high starch values (Table II). Wood et al (1980) reported that many commercial amylases have considerable hemicellulase activity. The effect of a hemicellulase would be more pronounced in pericarp tissue, because of the higher levels of hemicellulose in the cell walls than in the whole grain, and could account for part of the high starch values obtained.

The testa fraction isolated by abrasive milling was contaminated with endosperm particles. This explained the high starch and protein values observed (Table II). Only small amounts of pericarp tissue were observed in the testa fraction. For the varieties without a pigmented testa (BTx3197 and ATx399 × RTx430), this fraction appeared to be primarily composed of peripheral endosperm and aleurone cells.

Phenol Analyses

Phenols as characterized by the vanillin, Folin-Ciocalteu, anthocyanidin pigments, and RDP assays are shown in Tables III–VI. The RDP values determined in this study were similar to values shown by Butler (1982b) for a group III sorghum (DeKalb BR69), which ranged from 0.2 to 1.4 during maturation of the kernel. The RDP is a relative measure of the average length of tannin molecules and does not measure the number of monomer units in each tannin molecule. No significant levels of phenols were extracted from the endosperm tissue of any of the sorghum varieties. Bound phenols that were primarily associated with cell walls existed in the sorghum endosperm (Doherty et al 1983), but they were not extracted with methanol, acidic methanol, or DMF.

Red (*RRYY*) Versus White (*RRyy*) Pericarp: Group I Sorghums

There was no difference in the levels of vanillin- or Folin-Ciocalteu-positive phenols present in the whole grain, pericarp, and testa of group I sorghums ($b_1b_1B_2B_2$) with red or white pericarps. In group I sorghums and other samples with low levels of phenols, tyrosine, present in the extract, may be a significant interference (Ring 1984). Tyrosine, with its aromatic hydroxyl group, reacts with the Folin-Ciocalteu reagent and any other reagent that relies on aromatic hydroxyl or reducing power to measure phenols. There was a large increase in the level of anthocyanidin pigments in the pericarp when *R* and *Y* were both dominant (red pericarp), but not when the pericarp was white (Table V). The samples grown at Halfway, Texas, had significantly more anthocyanidin pigments than those grown at College Station. Several anthocyanin pigments in red and white sorghums have been identified by Nip and Burns (1969, 1971). Group I sorghums did have small amounts of vanillin-positive material. These compounds most likely were leucoanthocyanidins or other flavonoid compounds that can react with vanillin (Starker and Howarth 1976, Hahn et al 1984).

Red (*RRYYB*$_1$-*B*$_2$-*ss*) Versus White (*RRyyB*$_1$-*B*$_2$-*ss*) Pericarp: Group II Sorghums

Group II sorghums with a red pericarp were higher in vanillin- and Folin-Ciocalteu-positive phenols than those with a white pericarp (Tables III and IV). In general, however, the vanillin and Folin-Ciocalteu assay phenols showed the same trends. This was expected as tannins are the major phenols present in sorghums with a pigmented testa. The group II sorghums behaved as described by Butler and Price (1977), with tannins being extractable in significant amounts only with acidic methanol. Tx2566 (*R-Y-B*$_1$-*B*$_2$-*ss*-) had more anthocyanidin pigments than Early Hegari (*R-yy-B*$_1$-*B*$_2$-*ss*-) in the pericarp and testa fractions. Presence of a testa layer increased the level of the anthocyanidin pigments in the pericarp and testa fractions (Table V) of both Tx2566 and Early Hegari compared with the corresponding group I sorghums. This did not appear to be a result of contamination of pericarp by testa. The RDP of the tannins in the white pericarp was larger than those in the red pericarp for sorghums grown at College Station in 1983. Some of these differences may be caused by a dilution of tannins by flavonoid monomers. The *R* and *Y* genes did not affect the length (RDP) of the tannins in the testa of group II sorghums.

TABLE I
Genetic Constitution and Appearance of Six Sorghum Varieties

Variety	Genotype	Group	Testa	Pericarp Color	Pericarp Thickness	Appearance
BTx3197	*RRyyiib*$_1$*b*$_1$*B*$_2$*B*$_2$*SSzz*	I	absent	white	thick	chalky white
ATx399 × RTx430	*RRYyIib*$_1$*b*$_1$*B*$_2$*b*$_2$*SSZz*	I	absent	red	thin	brownish red
Early Hegari	*RRyyiiB*$_1$*B*$_1$*B*$_2$*B*$_2$*sszz*	II	present	white	thick	purplish white
Dobbs	*RRyyiiB*$_1$*B*$_1$*B*$_2$*B*$_2$*SSzz*	III	present	white	thick	brownish white
Tx2566	*RRYYIIB*$_1$*B*$_1$*B*$_2$*B*$_2$*ssZZ*	II	present	red	thin	brownish red
ATx623 × SC0103	*RRYyIiB*$_1$*b*$_1$*B*$_2$*B*$_2$*SSzz*	III	present	red	thick	brownish red

Red (*RRYYB$_1$-B$_2$-S-*) Versus White (*RRyyB$_1$-B$_2$-S-*) Pericarp: Group III Sorghums

Dominant *R* and *Y* increased the levels of both tannins and phenols present in the pericarp of group III sorghums (Table III and IV). The *R* and *Y* genes did not affect the level of tannins in the testa fraction of group III sorghums. However, there were considerably more Folin-Ciocalteu-positive phenolic compounds extracted with methanol from the testa fraction of Dobbs (*R-yy-B$_1$-B$_2$-S-*) than from the testa fraction of ATx623 × SC0103 (*R-Y-B$_1$-B$_2$-S-*). Only a small part of this increase appeared to be caused by anthocyanidin pigments (Table V). Amounts of anthocyanidin pigments extracted with methanol from the pericarp and those extracted with acidic methanol from the testa were greater in sorghums with a white pericarp. Neither the anthocyanidin pigments extracted with acidic methanol and DMF from the pericarp nor those from the testa were affected by pericarp color (*R* and *Y*). The RDP of the tannins of group II sorghums was not affected by *R* and *Y* (Table VI).

TABLE II
Composition of Whole Grain, Pericarp, and Testa Fractions (dry wt basis) of Five Sorghum Cultivars Grown at Halfway, TX, in 1981

	Protein[a,b] (%)			Total Starch[c] (%)		
Variety	Whole Grain	Pericarp	Testa	Whole Grain	Pericarp	Testa
BTx3197	12.0	4.7	13.8	76.7	42.5	53.1
ATx399 × RTx430	13.4	8.6	14.3	73.8	41.0	55.9
Early Hegari	11.8	4.8	11.4	75.4	44.1	52.2
Tx2566	13.2	13.8	14.3	69.1	32.9	36.7
ATx623 × SC0103	13.1	6.6	13.9	68.7	37.2	47.5

[a] Least significant difference is 1.6 at $\alpha = 0.05$.
[b] N × 6.25.
[c] Least significant difference is 3.6 at $\alpha = 0.05$.

Group I (*b$_1$b$_2$B$_1$B$_2$*) Versus Group II (*B$_1$-B$_2$-ss*) Sorghums

Group II sorghums contained more vanillin-positive phenols in the acidic methanol and DMF extracts of the whole grain, pericarp, and testa than group I sorghums, but there was not much difference in vanillin-positive phenols in the methanol extract of group I and II sorghums. Group II sorghums were higher in Folin-Ciocalteu phenols than the corresponding Group I sorghums with the same pericarp color.

Group II (*B$_1$-B$_2$-ss-*) Versus Group III (*B$_1$-B$_2$-S-*) Sorghums

A dominant *S* gene in the presence of B_1-B_2 increased the level of total phenols (Folin-Ciocalteu) and tannins (vanillin) in the whole grain as well as in the pericarp and testa tissues. This increased level

TABLE III
Values for Vanillin Assay in Catechin Equivalents (mg/100 mg) for Six Sorghum Varieties Extracted Sequentially with Methanol, Acidic Methanol (1% HCl) and Dimethylformamide (DMF)

	Whole Grain			Pericarp			Testa		
Variety	Methanol	Acidic Methanol	DMF	Methanol	Acidic Methanol	DMF	Methanol	Acidic Methanol	DMF
Halfway, TX, 1981									
BTx3197	0.08	0.06	0.07	0.08	0.07	0.07	0.11	0.15	0.10
ATx399 × RTx430	0.08	0.06	0.07	0.14	0.16	0.14	0.22	0.20	0.14
Early Hegari	0.14	0.24	0.11	0.16	0.32	0.12	0.08	1.57	0.21
Tx2566	0.05	0.24	0.13	0.19	0.66	0.20	0.25	2.17	0.24
ATx623 × SC0103	0.78	0.41	0.17	0.97	0.45	0.22	7.05	1.32	0.18
LSD[a]	0.05	0.08	0.02	0.06	0.09	0.06	0.40	0.07	0.04
College Station, TX, 1983									
BTx3197	0	0.13	0.14	0.16	0.05	0.11	0	0	0.16
ATx399 × RTx430	0	0	0.12	0.05	0	0.05	0	0	0.07
Early Hegari	0	0.59	0.21	0	0.76	0.21	0	5.73	0.42
Dobbs	1.08	1.22	0.19	0.62	0.87	0.06	5.52	3.76	0.39
Tx2566	0.43	1.31	0.21	0	2.69	0.45	0.40	6.72	0.35
ATx623 × SC0103	0.90	1.10	0.21	2.81	1.88	0.39	5.96	3.75	0.21
LSD[a]	0.10	0.13	0.04	0.31	0.29	...[b]	0.70	0.61	0.21

[a] Least significant difference for each column (solvent) $\alpha = 0.05$.
[b] Column not significantly different at $\alpha = 0.05$.

TABLE IV
Total Phenols Measured as Gallic Acid Equivalents by the Folin-Ciocalteu Assay (mg/100 mg) for Six Sorghum Varieties Extracted Sequentially with Methanol, Acidic Methanol (1% HCl), and Dimethylformamide (DMF)

	Whole Grain			Pericarp			Testa		
Variety	Methanol	Acidic Methanol	DMF	Methanol	Acidic Methanol	DMF	Methanol	Acidic Methanol	DMF
Halfway, TX, 1981									
BTx3197	0.05	0.06	0.03	0.06	0.08	0.05	0.14	0.08	0.10
ATx399 × RTx430	0.07	0.04	0.03	0.35	0.32	0.14	0.26	0.21	0.10
Early Hegari	0.04	0.23	0.10	0.07	0.33	0.10	0.13	1.10	0.35
Tx2566	0.08	0.22	0.10	0.49	0.68	0.23	0.58	1.52	0.45
ATx623 × SC0103	0.65	0.29	0.12	1.11	0.64	0.40	5.25	0.96	0.41
LSD[a]	0.07	0.04	0.02	0.05	0.01	0.04	0.26	0.05	0.03
College Station, TX, 1983									
BTx3197	0.15	0.19	0.07	0.09	0.16	0.08	0.24	0.24	0.10
ATx399 × RTx430	0.16	0.15	0.07	0.37	0.55	0.26	0.24	0.26	0.10
Early Hegari	0.14	0.47	0.13	0.13	0.58	0.15	0.34	2.43	0.48
Dobbs	0.31	0.61	0.18	0.28	0.70	0.21	4.54	1.66	0.34
Tx2566	0.18	0.67	0.17	0.58	1.69	0.41	0.67	3.37	0.60
ATx623 × SC0103	0.30	0.60	0.19	1.16	1.24	0.40	2.95	1.82	0.44
LSD[a]	0.03	0.07	0.01	0.03	0.05	0.01	0.11	0.64	0.03

[a] Least significant difference for each column (solvent) $\alpha = 0.05$.

of phenols occurred primarily in the compounds extractable with methanol. Sorghum varieties with a recessive *S* (*ss*) and B_1-B_2- actually had higher levels of tannins and phenols extracted with acidic methanol. Tannins present in the testa layer of group II and III sorghums had the same RDP, but they had different extractabilities (Table III). Group III sorghums had two types of tannins, one soluble only in acidic methanol and the other extractable in methanol, whereas the tannins of Group II sorghums were soluble only in acidic methanol. The RDP tended to increase with each sequential extraction, the DMF extract being the largest. The difference in RDP between the tannins extractable with methanol and acidic methanol was not statistically significant. Butler (1982b) has suggested that the tannins of group II sorghums may increase in length during maturation and lose some of their ability to react with protein. There was no difference in RDP between group II and III tannins of the sorghums used in this study. The only difference between these tannins appeared to be their extractability. Group II sorghum tannins appeared to have the same RDP but may be physically or chemically bound in the grain.

Effect of Environment on Phenols

The samples grown at College Station were considerably higher in total phenols and vanillin-positive material than those grown at Halfway. The increase in Folin-Ciocalteu- and vanillin-positive phenols observed in the sorghum grown at College Station was accompanied by a decrease in the levels of anthocyanidin pigments in all the tissues. This suggested that a portion of the pigments in the grain may be converted to tannins and other phenols under some environmental conditions. Some differences were also observed in the RDP of tannins in the testa of sorghums grown at Halfway that were not observed in the varieties grown at College Station. For group III sorghums grown at College Station, tannins extracted with methanol had a lower RDP and those extracted with acidic methanol and DMF had a higher RDP. At College Station, the grain matured under wet, humid conditions that were ideal for growth of molds, and the grain was slightly weathered. The dry conditions at Halfway produced very high quality grain. The increased phenol content of grain grown at College Station may be a reaction of the developing seed to protect itself from fungal attack. Sunlight may also affect phenol levels in the grain. Raab (1983) reported that some sorghum varieties had a much lighter color when the grain matured under a bag. In addition, the synthesis of some phenols is known to be controlled by light (Hrazdina and Parsons 1982).

Pronase Inhibition

The pronase inhibition of the whole grain, pericarp, and testa is shown in Table VII. The whole grain of group III sorghums showed more inhibition than the group II sorghums and the group I sorghum ATx399 × RTx430. For the other group I sorghum, BTx3197, inhibition for the ground whole grain was the same as for group III sorghums. This was in contrast to the results of Hahn et al (1982), where the group I sorghums had significantly higher digestibilities than group II or III sorghums using the same enzyme system. The reason BTx3197 showed this amount of inhibition was not readily apparent. It seemed that there were compounds other than tannins and phenols in this sorghum variety that could inhibit pronase. The inhibition of the pericarp and testa layer was signif-

TABLE V
Anthocyanidin Pigments Measured as Absorbance Per Gram of Tissue of 1 ml of Extract in 10 ml of 1% HCl in Methanol; Six Sorghum Varieties Extracted Sequentially with Methanol, Acidic Methanol (1% HCl), and Dimethylformamide (DMF)

	Whole Grain			Pericarp			Testa		
Variety	Methanol	Acidic Methanol	DMF	Methanol	Acidic Methanol	DMF	Methanol	Acidic Methanol	DMF
Halfway, TX, 1981									
BTx3197	0.48	0.33	0.12	1.41	1.13	0.95	2.40	1.35	1.30
ATx399 × RTx430	1.19	0.77	0.58	8.10	8.90	2.26	6.93	4.19	0.72
Early Hegari	0.25	1.72	0.28	4.18	5.81	2.41	1.39	4.83	0.36
Tx2566	1.22	2.42	0.05	11.39	10.75	1.32	8.76	10.33	1.09
ATx623 × SC0103	1.77	2.32	0.10	11.98	12.05	5.69	9.37	5.29	1.99
LSD[a]	0.36	0.47	...[b]	0.91	1.20	1.91	0.49	0.43	0.86
College Station, TX, 1983									
BTx3197	0.26	0.30	0.69	0.50	0.85	1.03	0.64	0.67	0.88
ATx399 × RTx430	0.46	0.67	0.78	1.36	2.50	1.17	0.84	0.98	0.98
Early Hegari	0.50	0.61	0.80	1.05	1.39	0.96	1.41	1.72	1.19
Dobbs	0.76	0.91	0.86	1.36	1.75	1.37	1.44	1.70	1.27
Tx2566	0.57	0.97	0.90	3.14	4.65	1.31	1.79	3.06	1.35
ATx623 × SC0103	0.38	0.66	0.93	1.15	1.76	1.21	1.37	1.44	1.31
LSD[a]	0.03	0.04	0.07	0.09	0.17	...[b]	0.09	0.11	0.14

[a]Least significant difference for each column (solvent) $\alpha = 0.05$.
[b]Column not significantly different at $\alpha = 0.05$.

TABLE VI
Relative Degree of Polymerization of Tannins for Four Sorghum Varieties Extracted Sequentially with Methanol, Acidic Methanol (1% HCl) and Dimethylformamide (DMF)

	Whole Grain			Pericarp			Testa		
Variety	Methanol	Acidic Methanol	DMF	Methanol	Acidic Methanol	DMF	Methanol	Acidic Methanol	DMF
Halfway, TX, 1981									
Early Hegari	2.50	1.53	4.75	1.60	1.76	5.24	1.41	1.33	7.01
Tx2566	1.81	1.47	2.51	3.34	4.05	2.82	2.64	1.40	6.61
ATx623 × SC0103	1.43	1.07	5.02	1.31	3.40	3.66	1.20	2.29	9.41
LSD[a]	1.59	...[b]	1.47	0.39	0.76	...[b]	0.14	0.19	2.15
College Station, TX, 1983									
Early Hegari	...	0.88	3.21	...	1.56	2.10	...	0.70	3.52
Dobbs	0.55	0.76	2.70	0.67	1.16	4.06	0.50	0.67	...
Tx2566	0.92	1.19	12.38	...	1.03	11.30	...	0.90	4.65
ATx623 × SC0103	0.67	0.82	7.52	0.71	0.88	6.49	0.60	1.06	3.73
LSD[a]	0.58	...[b]	...[b]	0.29	0.49	5.99	0.62	0.19	1.88

[a]Least significant difference for each column (solvent) $\alpha = 0.05$.
[b]Column not significantly different at $\alpha = 0.05$.

TABLE VII
Percent Inhibition of Pronase Hydrolysis of Casein by Sorghum Varieties Grown at College Station, Texas, in 1983

Variety	Group	% Inhibition[a,b]		
		Whole Grain	Pericarp	Testa
BTx3197	I	19.3	13.7	3.0
ATx399 × RTx430	I	12.3	13.3	9.0
Early Hegari	II	15.7	10.0	11.0
Tx2566	II	13.3	9.3	13.0
Dobbs	III	19.3	17.3	18.7
ATx623 × SC0103	III	19.3	22.0	28.0

[a]LSD = 5.3 at $\alpha = 0.05$.
[b]Casein control has 0% inhibition (digestibility = 100%).

icantly higher for the group III sorghums than for the other varieties. The inhibition of the group II sorghums was not different from the group I sorghums for any of the tissue fractions. This supported reports in the literature that the antinutritional effects of group III sorghums were absent in some group II sorghums (Butler 1982b).

CONCLUSIONS

R, *Y*, B_1, B_2, and *S* genes influenced both the level and location of phenolic compounds in the sorghum kernel. Each gene influenced the phenols in both the pericarp and testa in varied degrees. *R* and *Y* genes primarily influenced the phenols in the pericarp, especially the anthocyanidin pigments. The anthocyanidin pigments increased when both *R* and *Y* were dominant (red pericarp). Dominant B_1 and B_2 genes controlled the presence of a pigmented testa layer and tannins in sorghum. Tannins were found in significant amounts in the pericarp as well as the testa. Vanillin-positive compounds (leucoanthocyanidins) were found in the pericarp and testa of group I sorghums. *S*, when dominant in the presence of B_1 and B_2, increased the level of phenols in both the pericarp and testa layers. The level of anthocyanidin pigments was similar in red and white pericarps of group III sorghums. This could explain why group III sorghums are brown regardless of pericarp color. Differences in extractability of tannins from group II and III sorghums indicate that there were two groups of tannins present in group III sorghums, but only one group present in group II sorghums. The more easily extracted tannins (group III) tended to have a lower RDP. Testa and pericarp fractions from group III sorghums inhibited the action of pronase more than these fractions from group II sorghums. This may have resulted from an increased specificity or an increased solubility of the methanol-extractable tannin that enabled them to bind more readily with protein.

There were many factors that influenced the color and presumably the phenols of sorghum grain. Many of these, such as glume and plant color, are not well understood. The genes discussed in this paper (*R*, *Y*, B_1, B_2, and *S*) are the major and best understood genes that influence grain color. The intensifiers (I), I_p (purple testa) and the sun red gene, are known to affect grain color, but their effects are not understood. There is also variability in the levels of tannins and phenols in sorghum with similar genotypes. The six varieties used in this study were chosen with as many as possible of the other influences remaining the same, so that the differences observed would be primarily caused by the influence of the *R*, *Y*, B_1, B_2, and *S* genes. More sorghum varieties with these genotypes need to be analyzed to more clearly determine the effect of genotype and environment on the phenolic compounds of sorghum.

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