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# Sorghum Polyphenols and Bird Resistance

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Chemical, biochemical, and bird-repellent properties of polyphenols from a wide selection of sorghum varieties were evaluated. Several were evaluated in four stages of seed maturation. Also, the influence of molecular characteristics of 15 Sephadex LH-20 fractions were studied. Changes in sorghum polyphenol properties during maturation parallel those in other plant products. The molecular characteristics that influence chemical and biochemical properties of condensed tannins also appear to affect the degree of repellency to *Quelea quelea*. These studies may lead to development of sorghums with improved bird-repellent and nutritional characteristics.

Among North Americans and Europeans, sorghum is one of the least known of the world's major crops. Its primary use in the developed countries is almost exclusively as a feed grain and forage crop. But in some parts of the world (e.g. India, the Sahelian zone of Africa, the Near East, and Middle East) sorghum is a staple food crop and forms the basis of nourishment for much of the human population (Martin 1970). In 1977 sorghum was grown on 44 million hectares, of which 37 million hectares were in developing countries (FAO 1978). The reliance on sorghum as a staple food crop in many areas is due to its ability to withstand relatively harsh ecological and growing conditions (Doggett 1970). In some arid regions (e.g. the Sahel of Africa) it is the only cereal grain that can be grown with reasonable success.

Like all crops, sorghum is affected by a number of pests, and granivorous (grain-eating) birds are among the more serious. The red-billed weaver (*Quelea quelea*) of Africa is, perhaps, the most classic example. It has the distinction of being the most numerous and possibly the most destructive bird in the world, comparable only to locusts in the amount of damage it causes. The *Quelea* population base in Africa is estimated to be between $10^9$ and $10^{11}$ (Crook and Ward 1968). Other species in other parts of the world likewise cause significant losses in sorghum plantings. Blackbirds, starlings, and sparrows are the major problem birds in North America. In southern Asia, sparrows, parakeets, crows, mynas, and several others cause havoc to sorghum crops (Rachie 1970). Eared doves, blackbirds, sparrows, parakeets, and parrots are the major problem species to sorghum growers in several Latin American countries. Hence, a great deal of interest is turned towards developing some means of alleviating the bird damage problem.

In areas such as Africa and many of the developing countries, the risks of such losses to birds are high. Agricultural practices are more labour intensive than capital intensive, hence, damage control technologies should be suited to these situations. Because chemical repellents or avicides are expensive, often difficult to obtain in developing countries, and require special equipment for application, it would seem more practical to place emphasis on building an ability to resist bird depredation into the genetic composition of the plants (Cummings 1976). Much effort has been directed to this end in sorghum. In fact, bird damage problems are often so severe that development of bird-resistant (BR) inbreds and hybrids became an integral part of many sorghum research programs during the 1960s and early 1970s (McMillian et al. 1972).

Bird resistance in sorghum has been attributed to several characteristics including the presence of stiff lemma awns (Boyd et al. 1965), lax panicles (Voight 1966; Perumal and Subramaniam 1973), and high tannin content (Fuller et al. 1966; Niehaus 1966; Tipton et al. 1970; Niehaus and Schmidt 1970). High tannin content is the characteristic most often associated with bird resistance and many data have been presented to support this relationship (Harris 1969; Tipton et al. 1970; McMillian et al. 1972). Several sorghum lines with high tannin content have been developed and promoted on the basis of their ability to resist depredation by birds. In fact, many of these bird-

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resistant sorghums are used extensively in those areas of the world where the risk of such losses is high.

The benefits to be gained, however, do not come without a price. Other researchers have found that the polyphenolics (tannins) which produce astringency; and thereby repellency, also reduce the palatability, digestibility, and nutritional quality of many foods containing them (Chang and Fuller 1964; Harris 1969; McGinty 1969; Glick and Joslyn 1970; Schaffert 1972; Cummings and Axtel 1973). One author (Morton 1970 and 1978) has proposed that there is a link between a high incidence of esophageal cancer in certain areas and the usage of tannin-containing plants within these areas. The result is that high-tannin sorghums have a lower value on the export market (Anonymous 1978), and farmers who must produce them because of high bird-depredation risk, are at an economic disadvantage.

There is an obvious need for further research and development in this area. Historically, most bird-resistant sorghum research has been restricted to field evaluations of damage. In view of the above, it seems reasonable to suggest that this approach is inadequate and that the factors affecting both bird resistance and nutritional quality in sorghum must be looked at from a different viewpoint. The purpose of the present paper is to review knowledge of those factors, chemical and biochemical, which influence the bird-resistant and nutritional characteristics of sorghum, and to examine how they may be utilized in the development of varieties that will adequately fulfill the needs of sorghum farmers around the world.

## Classification of Sorghum Tannins

A review of the literature covering the past 20 years of sorghum research and development reveals a fascinating progression of understanding of the properties of polyphenolic tannins and their role in chemical bird resistance. For example, before 1970 most laboratories used tannic acid and other hydrolyzable tannins as standards for comparison in chemical and nutritional studies of sorghum tannins. Today, most agree that hydrolyzable tannins, if present, are found only in trace quantities, and that condensed tannins are responsible for the bulk of protein-binding activity (Bate-Smith and Rasper 1969; Strumeyer and Malin 1975). Although the term bitter has often been used to describe a taste property of sorghum polyphenols, most workers attribute bird resistance to the tactile astringent response. Astringency is that mouth-puckering sensation that results from coagulation of the proteins of the saliva and mucous epithelium when in contact with tannins (Joslyn and Goldstein 1964).

Condensed tannins are condensation products of flavan-3,4-diols with some incorporation of flavan-3-ols, primarily catechin or epicatechin. The biochemical activity of the polyphenol molecule depends on its ability to bind with various proteins. Such interactions are manifested in enzyme, fungi, and viral inhibition; in the tanning of hides; in lowered nutritional quality; and in the astringent taste response.

Flavan-3,4-diols are known to produce coloured anthocyanidins on heating with dilute acid (Ribereau-Gayon 1972). Several of these anthocyanidin generating compounds have been identified in various sorghum seeds. However, there has been a general tendency not to distinguish between those that condense to form tannins and those that serve only as pigments.

The use of chemical analyses to distinguish between these proanthocyanidin tannins and nontannins can be misleading. Even the use of the favoured vanillin assays will detect anthocyanins (Sarker and Howarth 1976) or any proanthocyanidin that contains an unsubstituted phloroglucinol or resorcinol structure (Ribereau-Gayon 1972) whether or not they readily condense into tannins.

Profisetinidin was found in the pericarp of Martin sorghum (Blessin et al. 1963) and propelaragonidin in the pericarp of a "commercial sorghum" (Yasumatsu et al. 1965) but neither of these proanthocyanidins was tested for protein-binding properties. Bate-Smith and Rasper (1969) identified the principal "tannin" of a Kafir variety as luteoforol or proluteolinidin, which is a flavan-4-ol. Tuey and Maslam (1977) reported the appearance of a red or pink-red pigment, stated to be mostly luteolinidin, as the green seeds begin to ripen. The authors of this paper and others (Strumeyer and Malin 1975) have found a similarly coloured pigment in the nontannin fraction from Sephadex LH-20 gel permeation chromatographic separation of polyphenolic extracts from BR (bird-resistant) sorghums. Unfortunately, all of Bate-Smith and Rasper's determinations of astringency were for the synthetic product of the borohydride reduction of eriodictyol and not an isolated natural tannin. Folin-Denis analyses which detect any phenols present (Goldstein and Swain 1963) were used for all of their tannin determinations.

The fact that Bate-Smith and Rasper (1969) reported luteoforol values for most sorghum samples, whereas only a few off-coloured

(bronze) seeds from their Kafir sample contained a testa, leads us to suspect that they were dealing with a nontannin pigment. Most investigators agree that a testa must be present for a variety to have significant quantities of tannin (Harris 1969; Maxson et al. 1972; Price and Butler 1977). Conversely, pericarp colour or pigmentation is not necessarily related to tannin content or biochemical activity (Thayer et al. 1957; Blessin et al. 1963; Stephenson et al. 1968; Harris 1969; Damron et al. 1968; Maxson et al. 1972 and 1973; Mabbayad and Tipton 1975, Nelson et al 1975). Red pericarp colour is controlled by two independent genetic factors Y and R (Wanjari and York 1972; Kambal and Bate-Smith 1976) while $B_1B_2S$ controls the brown pericarp, the presence or absence of a pigmented testa, and tannin content (Maxson et al. 1972; Wanjari and York 1972).

All of this leads to the point that procyanidin chemistry is most likely involved in the bulk of the reported chemical, biochemical, and nutritional investigations of BR sorghums. This becomes apparent in reports of chemical investigations of testa-containing, high-tannin sorghum varieties. Strumeyer and Malin (1975) isolated the tannin fraction from Leoti and Georgia 615 varieties and found it to be exclusively procyanidin in nature. Haslam (1977) also found the tannins of NK 300 (Haslam, personal communication) to be procyanidin in nature.

### Shape and Conformational Influences

Molecular shape is an important factor in determining the properties of procyanidin polymers (Quesnel 1968). Haslam and co-workers have made important contributions to the understanding of molecular configuration in procyanidin tannins. They categorized plants containing procyanidins into four groups based on their "procyanidin fingerprint." The configuration of NK 300 tannins was determined to be associated with (+)-catechin, procyanidin B-1, and associated oligomeric procyanidins. Inspection of molecular models has facilitated the prediction of preferred shapes (Fletcher et al. 1977; Haslam 1977). On this basis, sorghum procyanidin would be a right-hand helical structure. The central core would be composed of rings A and B of (−)-epicatechin with ring C projecting laterally from this core and with a terminal (+)-catechin unit.

### Molecular-Size Influences

The size of condensed tannin molecules has long been known to influence their protein-binding activity (Goldstein and Swain 1963; Ribereau-Gayon 1972). One tannin molecule binds with two or more peptide groups, primarily through hydrogen bonding, and thus forms cross-links between protein chains (Calderon et al. 1968) and causes precipitation. Flavan monomers and dimers are apparently too small, whereas highly polymerized tannins are either too insoluble or too bulky to fit between suitably oriented protein molecules. Significant protein-binding activity is usually associated with oligomers containing 3–10 monomeric residues (Goldstein and Swain 1963; Roux 1972). Larger-sized molecules are referred to as polymers. Up to a certain molecular weight, as the molecular size of the procyanidin oligomer increases so does astringency. Molecular size also influences the ability of tannins to tan hides (White 1956), to inhibit enzymes (Byrde et al. 1960), and to reduce the nutritive value of foods (Martin-Tanguy et al. 1977).

### Changes in Sorghum Tannin during Ripening

Goldstein and Swain (1963) have studied the changes in tannins of several fruits during ripening. They concluded that loss of astringency upon ripening is probably related to increased tannin polymerization with resulting reduced solubility and protein-binding capacity. Similar changes in ripening sorghum seed have been widely referred to, until recently, as a "disappearance of tannin." Results of sorghum maturation studies indicate a general increase in tannin content through the immature stages and then, with few exceptions, a decrease or plateau for the fully ripened grain (Tipton et al. 1970, Johari et al. 1977, Davis and Hoseney 1979b; Price et al. 1979; Bullard et al. 1979b). In our studies of eight BR sorghum varieties in four stages of maturity (Bullard et al. 1979b) we observed an increase in vanillin, Folin-Denis, and leucoanthocyanin values (Table 1) through the mid- to late dough stages and a subsequent decline as maturity was reached. Additional tests, which measure protein-tannin interactions directly ($\alpha$-amylase inhibition and protein binding) or indirectly (preference testing with *Quelea*), revealed much the same pattern. The *Quelea* preference means were consistent with those of other chemical and biochemical assays. Individual t-tests indicated significant reductions in *Quelea* preference for the immature stages but analysis of variance did not show any overall differences. A combination of animal variations in behavioural tests and the fact that five of the eight varieties were high in tannin (discussed later) probably accounts for the reduced sensitivity (Bullard et al. 1979b).

The overall decrease (though not significant at $p = 0.05$) in vanillin/Folin (V/FD) and vanillin/

Table 1. Means for chemical and biochemical assays of tannin values in eight developing and ripening bird-resistant (BR) sorghum varieties.[1]

| | Chemical assays[2] | | | | | Biochemical assays[3] | | |
|---|---|---|---|---|---|---|---|---|
| Stage | Vanillin-$H_2SO_4$ (CE) | Folin-Denis (CE) | Leucoanthocyanin (Cyn E) | V/FD (%) | V/LA (%) | Protein binding (TAE) | α-amylase inhibition (%) | *Quelea* preference (%)[4] |
| Milk | 0.18*a* | 0.46*a* | 0.30*a* | 39.1*a* | 60.0*a* | 1.09*a* | 19.9*ab* | 38.2*a* |
| Light dough | 0.31*b* | 0.76*b* | 0.66*b* | 41.8*a* | 46.9*a* | 1.37*ab* | 23.7*ab* | 34.2*a* |
| Firm dough | 0.43*b* | 0.85*b* | 0.74*b* | 50.6*a* | 58.1*a* | 1.47*ab* | 24.2*a* | 30.8*a* |
| Mature | 0.16*a* | 0.47*a* | 0.45*ab* | 34.0*a* | 35.5*a* | 1.61*b* | 11.8*b* | 37.5*a* |

[1]Aliquots of combined acetone, methanol, and 90% methanol extracts of samples from eight BR sorghum varieties in four stages of maturity. Discussed in detail in Bullard et al. (1979c).

[2]Chemical assays of extract aliquots conducted by the respective procedures of Hills and Swain (1959) expressed in catechin equivalents (CE) for vanillin-$H_2SO_4$ and Folin-Denis assays and cyanidin equivalents (Cyn E) for leucoanthocyanin assay.

[3]Biochemical assays of extract aliquots conducted by following procedures: α-amylase inhibition in percent by Barnes and Blakely (1974); protein binding in tannic acid equivalents (TAE) by Hagerman and Butler (1978); *Quelea* preference in percent by Bullard and Shumake (1979).

[4]Preference response of *Quelea quelea* when given a choice between millet having surface-coated extract and control millet; percent preference = ((test food consumed)/(test + control food consumed)) × 100.

NOTE: Values followed by the same letter are not significantly different at the 0.05 level of Duncan's Multiple Range Test.

leucoanthocyanin (V/LA) from the firm dough to the mature stage, indicates that polyphenols in sorghums grow larger in molecular size after ripening begins (Table 1). The V/FD ratio has been used by Goldstein and Swain (1963) as an indicator of polyphenol molecule size. Vanillin reagent reacts in an approximately stoichiometric manner with unsubstituted phloroglucinol groups in tannins, and Folin-Denis reagent reacts nonstoichiometrically with hydroxyl groups, therefore the ratio can be used as an indicator of molecular size. However, the V/LA value is often used because both reagents react with flavanols, whereas the Folin-Denis reagent also reacts with nonflavan phenols. We favour the V/FD because of the problems with the leucoanthocyanin analysis (Lewak 1968) and because only sorghum in which the polyphenols are predominantly flavanols are involved (Bullard et al. 1979b).

The increasing V/FD values from the milk stage through the firm dough stage are consistent with ongoing synthesis of vanillin-reactive molecules from smaller phenols. The Folin-Denis reagent would be reactive with all intermediates, whereas vanillin could not react until phloroglucinol-containing molecules have been synthesized.

This pattern in developing and ripening sorghum seeds is consistent with the findings of Haslam (1977). He observed that as chlorophyll forms in the seed coat of sorghum, procyanidins are rapidly synthesized initially and then remain approximately constant until luteolinidin pigmentation occurs. Subsequently, a decrease in flavan-3-ol and associated co-metabolites is seen as relatively insoluble higher molecular weight polyphenols are formed.

## Group Chemical Classification of Sorghums

However, the foregoing description of the properties of sorghum tannins is not a complete picture. Cummings and Axtel (1973) and more recently Price et al. (1978) have categorized sorghums into three groups (I, II, or III) on the basis of differences between vanillin and modified vanillin assays. The two assays produce similar values for tannins in group III sorghums whereas the modified vanillin values are much higher than the unmodified vanillin for those varieties included in group II. Group II sorghums are of special interest because, in spite of the presence of a coloured testa and of tannin, they do not appear nutritionally inferior to low-tannin varieties (Cummings and Axtel 1973; Oswalt 1975; Hartigan 1979).

We have conducted *Quelea* preference tests and modified vanillin analyses and obtained Sephadex LH-20 gel permeation chromatographic profiles on 15 BR varieties with different genetic backgrounds (Bullard et al. 1979a). There was a significant negative correlation (r = -0.88) between food preference and CE by the modified vanillin assay of Maxson and Rooney (1972) as modified by Price et al. (1978) and between preference and peak area for tannins (r = -0.66) in the chromatographic profiles. However, there were definite inconsistencies in some varieties until they were grouped. With few exceptions, the intervarietal properties of group II and group III sorghums were similar whereas intragroup properties were different. Varieties in groups I and II had very similar chemical properties, elicited similar preference response in *Quelea*, and both were different from group III. Chromato-

Table 2. Comparative chemical and biochemical assays of tannin activity in bird-resistant (BR) sorghum by group.[1]

| Variety | Group | Protein binding (TAE)[2] | Vanillin (CE)[3] | Folin-Denis (CE)[4] | Leuco-anthocyanin (Cyn E)[3] | V/FD (%) | V/LA (%) |
|---|---|---|---|---|---|---|---|
| Bravis-BR-ATG-051 | I | 1.62*fg* | 0.55*h* | 0.40*h* | 0.03*i* | 137.5*f* | 1375.0*a* |
| IS 3063c | I | 1.58*g* | 0.45*h* | 0.53*g* | 0.18*j* | 84.9*f* | 250.0*b* |
| TAM 2566 | II | 1.82*ef* | 0.05*i* | 0.23*j* | 0.06*hi* | 21.7*gh* | 83.3*cd* |
| IS 2266c | II | 1.80*efg* | 0.05*i* | 0.35*hi* | 0.11*h* | 15.2*h* | 45.4*d* |
| IS 2319 | II | 1.99*de* | 0.05*i* | 0.23*j* | 0.06*hi* | 21.7*gh* | 83.0*cd* |
| Hegari | II | 1.78*efg* | 0.10*i* | 0.24*j* | 0.06*hi* | 41.7*g* | 166.6*bc* |
| AKS-614 | III | 2.72*ab* | 2.65*c* | 1.35*b* | 0.93*b* | 196.3*cd* | 284.9*b* |
| RA-Bird-Go-68 | III | 2.90*a* | 4.05*a* | 1.55*a* | 1.12*a* | 261.3*a* | 361.6*b* |
| AR 3005 | III | 2.89*a* | 3.50*b* | 1.50*a* | 1.12*a* | 233.3*ab* | 312.5*b* |
| Funks G516 BR | III | 2.51*bc* | 2.25*de* | 1.24*bc* | 0.80*c* | 181.4*d* | 281.2*b* |
| BR-54 | III | 2.58*bc* | 2.45*cd* | 1.13*e* | 0.72*d* | 216.8*bc* | 340.3*b* |
| IS 2403c | III | 2.30*cd* | 1.50*g* | 0.88*f* | 0.50*f* | 170.4*de* | 300.0*b* |
| ROKY 78 | III | 2.48*bc* | 1.80*fg* | 1.23*cd* | 0.87*e* | 146.3*e* | 206.9*bc* |
| IS 2801c | III | 2.30*cd* | 1.70*fg* | 0.89*f* | 0.66*e* | 191.0*cd* | 257.6*b* |
| WGF | III | 2.60*ab* | 1.95*ef* | 1.15*de* | 0.73*d* | 169.6*de* | 267.1*b* |

[1]Extract obtained by shaking ground sample with absolute methanol for 20 min at 30°C (Price et al. 1978). Respective analyses conducted on duplicate extracts.
[2]Protein binding assay conducted by method of Hagerman and Butler (1978).
[3]Vanillin assay conducted by method of Price et al. (1978).
[4]Folin-Denis and leucoanthocyanin assays conducted by method of Hillis and Swain (1959).
[5]Values followed by the same letter are not significantly different at the 0.05 level of Duncan's Multiple Range Test.

graphic profiles indicated that very little tannin was extracted from group II sorghums in comparison to group III while variable quantities of nontannin pigments were present in both.

In another experiment, samples were extracted for 20 min at 30°C with methanol (Price et al. 1978) and vanillin, Folin-Denis, leucoanthocyanin, and protein precipitation assays were conducted on each extract. In Table 2, we again observe large differences between group II and III varieties, while values within the groups were similar. Since absolute methanol was used in a brief extraction, we would expect polymer extraction to be minimal and the V/LA value would decrease for the oligomers extracted (Goldstein and Swain 1963). The smaller V/FD and V/LA ratios indicate that the mean size of oligomers in the extracts of group II was larger than that for group III. Therefore, size of the extracted as well as the unextracted tannins (see modified vanillin analysis obtained under same conditions in Table 3) is one explanation for the differences in properties of groups II and III.

When results of the evaluation of ripening sorghum discussed earlier (Bullard et al. 1979b) were arranged by group, the values were similar for both group II and group III varieties during the immature stages. However, with the exception of the protein-binding values, they were different for the mature grain. Although these particular group II varieties had slightly lower chemical and biochemical activity than group III during the immature stages, this pattern is an encouraging indication that intermediate-sized oligomers with protein-binding qualities are being formed during seed development. Thus, there is a good possibility that sorghum can be developed that is both bird resistant and of acceptable nutritional quality by careful selection of genomes within group II varieties.

## Possible Explanations of Group II and III Differences

An attractive hypothesis for explaining the differences between groups II and III is that the biosynthetic process of group II tannin polymers goes to a higher overall degree of completion during ripening than does that of group III. Group III would still contain a substantial proportion of protein-binding oligomers present in the mature grain, while group II would not. However, in testing this hypothesis we found that molecular size was not the only factor involved. The 15 mature grain samples discussed earlier, after vanillin analyses were reextracted for 3 h at 40°C with 50% methanol, which is designed to extract large molecules (Goldstein and Swain 1963), and then reacted with vanillin reagent

Table 3. Determining if the influence of HCl in the modified vanillin analysis affects only tannin solubility.

| Variety | Group | Modified vanillin (CE)[1] | Vanillin (CE)[1] | 2nd extraction vanillin (CE)[2] | Total vanillin (CE)[3] |
|---|---|---|---|---|---|
| Bravis-BR-ATG-051 | I | 0.10 | 0.55 | 0.005 | 0.56 |
| IS 3063c | I | 0.70 | 0.45 | 0.080 | 0.53 |
| TAM 2566 | II | 0.55 | 0.05 | 0.005 | 0.06 |
| IS 2266 | II | 0.30 | 0.05 | 0.065 | 0.12 |
| IS 2319 | II | 0.50 | 0.05 | 0.070 | 0.12 |
| Hegari | II | 0.70 | 0.10 | 0.025 | 0.12 |
| AKS-614 | III | 1.80 | 2.65 | 0.045 | 2.70 |
| RA-Bird-Co 68 | III | 3.25 | 4.05 | 0.045 | 4.09 |
| AR 3005 | III | 2.15 | 3.50 | 0.085 | 3.58 |
| Funks G516 BR | III | 1.95 | 2.25 | 0.110 | 2.36 |
| BR-54 | III | 2.05 | 2.45 | 0.070 | 2.52 |
| IS 2403c | III | 1.85 | 1.50 | 0.190 | 1.69 |
| ROKY 78 | III | 1.85 | 1.80 | 0.140 | 1.94 |
| IS 2801 | III | 0.90 | 1.70 | 0.075 | 1.78 |
| WGF | III | 1.60 | 1.95 | 0.035 | 1.98 |

[1]Extract obtained by shaking ground sample with absolute methanol (vanillin) or 1% HCl in methanol (modified vanillin) for 20 min at 30 °C (Price et al. 1978).
[2]Extract obtained by shaking previously extracted ground sample an additional 3 hrs at 40 °C with 50% methanol which is designed to extract difficultly soluble tannin polymers (Goldstein and Swain, 1963).
[3]Combined vanillin and 2nd extraction vanillin values.

(Table 3). When the two CE values from the two vanillin analyses for group II varieties were compared and analyzed by a paired t-test, they were still significantly lower than the modified vanillin CE values ($p = 0.01$). Similar comparisons with group III varieties yielded CE values that were significantly higher ($p = 0.007$) than the modified vanillin CE values. There were no differences in the two CE values for group I.

This indicates that the addition of 1% HCl in methanol for modified vanillin extracts affected more than just molecular size influences on tannin solubility. Important differences in starch (Davis and Hoseney 1979a), protein (Tamir and Alumot 1969), and cell wall polysaccharides (Goldstein and Swain 1963) may differentially influence binding with fragments of ground grain (Blessin et al. 1963). For example, Loomis and Battaile (1966) report that in addition to hydrogen bonding, phenols combine irreversibly with proteins by oxidation followed by covalent condensations. Price et al. (1979) have advanced a hypothesis that tannin vesicles become disrupted near maturity in group II sorghum grains causing enzyme polymerization and irreversible binding to some components of the pericarp.

Thus, there is a possibility that some or all of these factors account for differences in tannin properties between groups II and III. From our experience, another likely source of differences is based on the proanthocyanidin composition of the tannins. This, in turn, most likely affects molecular shape, which, according to Quesnel (1968), is at least as important as size. For example, Bate-Smith (1975) observed that prodelphinidins, which are second to procyanidins in frequency of distribution among plants (Thompson et al. 1972), are much more astringent than procyanidins. The chemical nature of proanthocyanidin monomers could also influence ultimate molecular size by affecting the rate and degree of tannin biosynthesis either enzymatically by polyphenol oxidases (Weinges 1964) or thermodynamically through the biogenetic process proposed by Haslam (1974). We have seen some indication that the peak of tannin activity occurs earlier in group II than in group III varieties.

Without exception, all of the group III methanol extracts of mature grain developed a scarlet colour during leucoanthocyanin analyses while those for group II were light brown. Acidic methanol (1% HCl) extracts of group II sorghums varied in colour from the same method of analysis; two were scarlet, two reddish-brown, and one brown. This is a good indication that not all sorghum tannins are exclusively procyanidin in composition and that substantial variations in composition can occur. In addition there are variations in testa colour (York 1976) which further strengthen this possibility.

Researchers at the University of Arkansas Agronomy Department, which supplied most of the BR varieties for the tests described, observed that testas of group II varieties AR 3009 and TAM 2566 had a different colour response in bleach tests than any of the group III varieties examined. They have also observed microscopic differences between the testas of varieties from groups II and III (York, personal communication).

## Future Prospects for Sorghum Development

There are many unknown properties which differentiate the group II and III sorghums. There are several literature references to varieties which have resisted bird depredation in the field and demonstrated good nutritional quality in feeding or digestion trials (Thayer et al. 1957; Damron et al. 1968; Harris et al. 1970; Mabbayad and Tipton 1975; Nelson et al. 1975; Oswalt 1975; Tipton et al. 1975). Similarly, no or very little enzyme-inhibition activity has been observed in varieties with a pigmented testa and/or sizeable levels of tannins (Maxson et al. 1973; Daiber 1975). One of the group II varieties tested in this laboratory (TAM 2566) has performed well under heavy bird depredation in Arkansas during the past five years (York, personal communication). Others tested did not withstand severe depredation in Puerto Rico this past winter (1978/79). A better understanding of the differences in polyphenolic composition and the biosynthetic process by which the active polyphenols are produced in sorghum will provide direction in the genetic selection of optimal characteristics for both the desireable bird-resistant qualities in immature seeds and nutritional qualities in the mature grain product.

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