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**WORK SHEET**

# The Role of Polyphenols in the Utilization of ICRISAT-mandated Grain Crops and Applications of Biotechnology for Improved Utilization

**L.G. Butler**[1]

***Abstract***

*The productivity of sorghum is to some extent dependent upon chemicals in the plant tissue that confer defense mechanisms against herbivores, fungal pathogens, and parasitic weeds. However, these defense chemicals limit the utilization of sorghum as human food. Cultivars that lack these defense mechanisms are often too vulnerable to yield losses for their widespread cultivation. An important group of defense chemicals in sorghum is the polyphenols, particularly flavonoids and their oligomers, the condensed tannins. Out of the rich variety of polyphenols produced by sorghum, individual components responsible for particular types of resistance are being identified. It is now possible to screen sorghums for optimum polyphenol composition and to improve the nutritional value without losing pest resistance. Methods are being developed to generate and propagate, in tissue culture, polyphenol-rich cultivars, and variants in respect of polyphenol metabolism that are suitable for wider cultivation.*

## Introduction

Polyphenols are compounds having two or more phenolic hydroxyl groups substituted on aromatic ring structures. Animals cannot synthesize polyphenols from nonaromatic precursors, but plants synthesize a wide variety of polyphenols. The major groups include flavonoids, tannins, and lignin. Phenolic acids are usually considered with the polyphenols although several of them have only a single phenolic hydroxyl group.

Plant polyphenols are not directly involved in metabolic pathways for growth and reproduction, and are therefore considered to be 'secondary' metabolites. Most polyphenols have no well-established function, but many are considered to play a role in defending the plant against the onslaught of herbivores, pathogens, and competitors. At least partly because of their lack of a clearly defined metabolic role, as well as the difficulty of isolating and characterizing them, relatively little is known about plant polyphenols, compared with other major components of plants.

Sorghum is capable of producing larger amounts of polyphenols than most other plants (Butler, in press). In addition to the condensed tannins for which its seeds are well known, sorghum has the capacity to produce a rich variety of flavonoids, some quite unusual and most not identified (Butler, in press). Millet would seem to be similarly dependent on chemical defenses, but millet has received less attention than sorghum in this respect. Even less is known about the polyphenols of the other crops of ICRISAT's mandate. The methodology developed and the results obtained from studies of sorghum polyphenols should be applicable to the other crops of ICRISAT's mandate.

Owing to the harmful effects that at least some polyphenols can produce when eaten, the capacity to synthesize large amounts of polyphenols tends to be eliminated during the domestication of the plant (Harborne 1982). Their absence improves the food

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value, but may render the plant vulnerable to herbivores, pathogens, or competitors to which its undomesticated ancestors were resistant. In the case of sorghum, cultivars containing low levels of polyphenols have been developed. They have desirable characteristics for use as food or feed, but they cannot be reliably cultivated in some areas because of vulnerability to various pests. Fortunately, even after long domestication, sorghum cultivars that retain considerable polyphenol-synthesizing defensive capability are still available. These cultivars are grown in areas where the incidence of pests is severe.

It is the task of the plant biotechnologist to devise appropriate methodologies, by selective genetic modification of the plant or, by innovative processing, to minimize or eliminate the antinutritional features of the polyphenols of crop plants, including those in ICRISAT's mandate, without compromising the resistance of the plants. Success in this endeavor would greatly expand the areas of Africa where the high-yielding and nutritionally acceptable sorghum could be produced. Similar benefits should be possible with other crops.

## Agronomic Effects of Plant Polyphenols

Production constraints of sorghum for which polyphenols contribute to resistance include grain losses due to predatory birds (McMillian et al. 1972), grain molding (Harris and Burns 1973), preharvest seed germination (Harris and Burns 1970), insect feeding on seedlings (Woodhead and Cooper-Driver 1979, Dreyer et al. 1981), and infection of seedlings by fungal pathogens and nonpathogens (Nicholson et al., in press).

We have recently found that the parasitic weed *Striga*, which is one of the major production constraints of sorghum and other crops in parts of Africa, utilizes a unique polyphenol exuded from sorghum roots as a germination signal. This polyphenol is the dihydroquinone of sorgoleone, a hydrophobic quinone that we have identified as the major component of the oily root exudate of sorghum root hairs (Chang et al. 1986). This chemical is implicated in signaling germination of *Striga* seeds. It has very low solubility in water and is chemically very unstable. Both these characteristics ensure the germination of any *Striga* seeds in close proximity to the host, and leave others dormant for subsequent seasons. Another agronomically beneficial effect of sorgoleone is inhibition of root elongation of certain plant competitors (Netzly and Butler 1986). The parasitic *Striga* apparently has adapted to the allelopathic defense chemical, utilizing its unstable precursor as a host-specific recognition signal. Strategies for controlling *Striga* by utilizing this polyphenol and other newly discovered chemical signals between *Striga* and its host are being developed.

## Utilization Constraints for which Polyphenols are Responsible

In some cultivars of sorghum (Scheuring et al. 1982) and millet (Reichert et al. 1980, Reddy et al. 1986), polyphenols contribute colors and/or tastes that are generally perceived to diminish palatability. With respect to nutritional value, condensed tannins of sorghum seed (absent in millet) are often associated with diminished weight gains and feed efficiencies of animals on experimental diets (Butler et al. 1986). It is usually assumed that the antinutritional effects associated with high-tannin sorghums are due to inhibition of digestion by the dietary tannin, but there is little solid evidence for this mechanism. Evidence is accumulating that the antinutritional effects may largely be due to inhibition of metabolic utilization of digested and absorbed foodstuffs (Mehansho et al. 1987). Some of the antinutritional effects of high-tannin sorghums do not appear to be due to the polymeric tannin molecules but to associated low molecular weight flavonoids more readily absorbed from the intestine (Mehansho et al. 1987).

From our recent work it is clear that polyphenols in the diet would have much more severe antinutritional effects if it were not for specific proline-rich, tannin-binding proteins present in the saliva of most animals that consume polyphenol-containing foods (Mehansho et al. 1987). These specialized proteins form strong complexes with dietary polyphenols as soon as they enter the digestive tract, diminishing but not eliminating their antinutritional effects.

## Applications of Biotechnology to these Constraints

The problem of antinutritional polyphenols in sorghum cannot be solved solely by developing cultivars low in total polyphenols. Such cultivars are available, but are too vulnerable to pests to be produced in some areas. In our laboratory we are attempting to identify specific polyphenols that are responsible for, or contribute to, particular agro-

nomic advantages or nutritional disadvantages. We would also like to determine the biochemical mechanisms of these polyphenol effects, and learn as much as possible about the biosynthesis of these specific polyphenols and how it is regulated. It may then be possible to use modern biotechnological methods to control or regulate the production of individual polyphenols, or groups of polyphenols, that are especially beneficial to the plant or especially harmful in the diet.

This approach can be successful only if different polyphenols are responsible for the beneficial agronomic effects and the harmful nutritional effects. Our early results suggest that this is the case, that the polyphenols responsible for antinutritional effects might be eliminated without leaving the crop too vulnerable. For example, the polymeric condensed tannins characteristic of the seed of 'bird-resistant' sorghums are virtually absent from the seed at the milk stage, when birds tend to do most of their damage. The 'bird resistance' seems to be due to shorter, lower-molecular-weight precursors of the polymers found in mature seed (Butler 1982). If these are the polymers that are largely responsible for the antinutritional effects of high-tannin sorghums, then inhibition or elimination of the enzyme that polymerizes the precursors should improve the nutritional value of the grain without leaving it vulnerable to birds. We have recently identified polyphenol oxidase as being at least partially responsible for the polymerization and we are seeking means of controlling its activity. Identification of a particularly crucial enzyme, especially one that is already available so that antibodies could be generated and used to identify in vitro translation products, open obvious opportunities to apply molecular biology to the problem.

Other examples of the association of specific polyphenols with particular agronomic benefits are the flavan-4-ols. These are monomeric flavonoids that are extremely rare. Sorghum is the only cereal (actually the only monocot) from which they have so far been positively identified. One of them, luteoforol, was reported from sorghum seeds by Bate-Smith (1969). We found that apiforol, which has one less hydroxyl than luteoforol, is relatively abundant and is present in leaf tissue as well as seed (Watterson and Butler 1983). Flavan-4-ols, which are inherited independently of condensed tannins (Watterson and Butler 1983), have no known antinutritional effects. Although our early work suggested a relationship between flavan-4-ol content and bird resistance (Subramanian et al. 1983), it was recently found that resistance to grain molding, rather than to birds, is associated with high flavan-4-ol content (Jambunathan et al. 1986). Even more recent studies, some with ICRISAT collaborators, have revealed that it is probably not the flavan-4-ols themselves that are responsibile for resistance to grain molds, but rather a series of flavanoids that are metabolically and chemically closely related to the flavan-4-ols. These components may be flavanones. In addition to polyphenols associated with agronomic benefits, we are attempting to identify polyphenol components responsible for antinutritional effects. The low-molecular-weight flavonoid fraction from quebracho was even more effective as an antinutritional agent in rat diets than was the high-molecular-weight polymer fraction (Mehansho et al. 1987). This experiment has not yet been done with sorghum, but preliminary observations suggest profound effects of high-tannin sorghums on metabolic parameters such as urinary volume. Reports of significant effects on phenolic detoxification enzymes (Sell and Rogler 1983) suggest that phenolic components of sorghum are absorbed from the digestive tract and strongly affect metabolism.

Our approach to applying biotechnology to the improvement of sorghum utilization begins with characterizing as completely as possible its individual polyphenol components with respect to their beneficial or harmful roles. This is presently one of our major emphases. Once particularly significant polyphenol components have been identified, we develop convenient assays for them so that breeders can utilize these results in plant-breeding programs. We are just beginning to be able to survey sorghum cultivars for the optimum complement of polyphenol components.

Our current approach to active manipulation of sorghum in order to optimize its polyphenol composition involves tissue culture of high-tannin genotypes. Most high-tannin sorghums grown as callus tissue produce massive amounts of polyphenols in intracellular membrane-bound inclusion bodies (Oberthur et al. 1983). Our observations suggest that these inclusion bodies eventually rupture and spill out their polyphenolic contents, which appear to be toxic to the cells that produced them. Out of the dying culture we rescue cells that can be cultured further, presumably because they have a diminished capacity to produce toxic polyphenols, or because they are more resistant to them. These cultures are of considerable interest with respect to their possible resistance to birds, molds, and other pests and the nutritional value. In 1986 we have learned how to

regenerate these calli into plantlets (Cai et al. 1987). The most difficult step is not regeneration but development of a viable root system. We are now evaluating, in the field, plants produced from the seed of hundreds of regenerated plants. This tissue-culture system appears to have advantages for isolation of variants that may have useful characteristics with respect to polyphenol production.

We are also interested in the possibility of modifying sorghum's complement of polyphenols by direct manipulation of its genome. Flavonoid metabolism in sorghum appears to differ considerably from other plants in that the unusual hydroxylation at the carbon 4, instead of carbon 3, seems to predominate in the monomeric flavonoids but not in the polymeric ones. A great deal of characterization of the enzymes of flavonoid biosynthesis must be carried out before attempting to manipulate the genome directly.

## Processing Technology for Polyphenol-rich Sorghums

Instead of modifying the sorghum plant so that it does not produce antinutritional polyphenols, considerable effort has gone into modifying the polyphenol-rich sorghum grain to eliminate or inactivate the polyphenols. The widely practiced process of decortication by pounding the grain in a crude mortar and pestle removes tannin rather effectively but nutrients are also lost (Chibber et al. 1978). Seeking a less labor-intensive and nutrient-depleting method for improving the nutritional value of high-tannin sorghum, we found that moistening the grain with aqueous alkalies several hours before utilization effectively eliminates the antinutritional effects of tannin. The treatment has no effect on the nutritional value of tannin-free sorghum (Price et al. 1979). Ammonia is the most effective alkali, probably because it penetrates the grain more rapidly than other alkalies. Nothing is removed from the grain by the treatment; presumably the polyphenols undergo oxidative polymerization into nutritionally inactive forms in the alkaline environment. The detoxification process requires water, for anhydrous ammonia is far less effective than dilute aqueous ammonia. The treatment is much more effective on whole grain than on ground grain. Apparently the deleterious interaction of the polyphenols with other components of the seed is enhanced by grinding the grain. The alkaline treatment darkens the color of most sorghum cultivars. Wild birds tend to choose ammoniated high-tannin sorghum over the untreated form of the same cultivar. These results have been summarized (Butler, in press) elsewhere.

Imbibition of high-tannin sorghum grain with aqueous alkalies is an important step in the traditional processing of high-tannin sorghums of eastern Africa. In Rwanda, Burundi, and some districts of Uganda most sorghum produced is very high in tannin. The predominant sorghum product of this region is a beer that contains all the grain. This product is fed to children before it ferments into beer. An early step in the production of traditional beer involves mixing the high-tannin grain with wood ashes, wetting the mixture and leaving it overnight. We found that this processing step with aqueous alkalies extracted from the ashes very effectively detoxifies the antinutritional effects of the high-tannin sorghum. Clearly, our newly discovered detoxification technology was merely the chemical rationalization of a traditional methodology.

Musalac®, a popular nontraditional food developed in Burundi for mothers and infants, contains 35% sorghum, in addition to maize, soy protein, dry milk solids, and sugar. The sorghum utilized is the locally produced type, high in tannin and not treated with wood ash in the traditional manner as is done for making beer. Our analyses indicated that the level of tannin in the final product was sufficiently high to produce significant antinutritional effects. This is particularly unfortunate in a food specially recommended for nutritionally vulnerable segments of the population. Following my recommendation, Musalac® has been prepared, on an experimental basis, from sorghum detoxified by the traditional wood-ash treatment. Tests are under way to determine if the anticipated improvement in its nutritional quality warrants the extra processing step.

I hesitated to include this alkali-detoxification story because it does not involve the use of advanced biotechnological methods, but rather the rational application of time-honored traditional methodology. I decided to include it because it contains an important message for the biotechnologist seeking to improve food quality and enhance utilization of traditional crops. We would do well to carefully examine traditional production and utilization methods. Although the traditional farmer or consumer may not be able to articulate a scientifically valid rationalization of why he does what he does, I have come to believe from this, and several other examples, that we have much to learn from methods maintained through many generations of sorghum production and utilization. Combining modern bio-

technology with traditional methodology may prove to be a powerful means of improving sorghum.


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