required and corn varieties that can tolerate these higher levels of EPTC must be used.

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Phenolics, Fiber, and Fiber Digestibility in Bird Resistant and Non Bird Resistant Sorghum Grain

Jess D. Reed

Differences between bird resistant (BR) and non bird resistant (NBR) sorghum grain in content of fiber and phenolics and relationships among phenolics, fiber, fiber digestibility, and inhibition of fungal cellulase were determined. Two methods for determining neutral-detergent fiber (NDF) were compared. The amylase procedure gave higher NDF values than the urea/amylase procedure. This difference was reflected in lower content of insoluble procyanidins in NDF prepared by the urea/amylase procedure. A newer method based on precipitation of soluble phenolics with ytterbium acetate was highly correlated with the vanillin HCl method. All phenolic components were significantly and negatively correlated with fiber digestibility. There was an inverse relationship between soluble phenolics and insoluble procyanidins in BR varieties. Measurement of soluble phenolics may give incomplete analysis of inhibitory phenolics. Measurements of insoluble procyanidins and/or lignin should be included in studies on the nutritive value of sorghum grain.

Sorghum is an important crop in the semiarid areas of Africa where birds are a major pest (Bullard and York, 1985). Bird resistance is related to the presence of procyanidins in the grain (Gupta and Haslam, 1980), and sorghum improvement programs are selecting bird resistant varieties. However, procyanidins in sorghum decrease nutritive value by complexing protein, carbohydrates, and minerals (Butler, 1982).

Soluble procyanidins are measured by the vanillin/HCl method (Price et al., 1978; Gupta and Haslam, 1980). A new gravimetric method for measuring soluble phenolics by precipitation with ytterbium acetate has been developed (Reed et al., 1985). This study applies the method to sorghum grain and compares it to the vanillin/HCl method.

Sorghums have been grouped on the basis of content and solubility of procyanidins (Price et al., 1978). Group I sorghums are non bird resistant and contain low quantities of vanillin-reactive phenolics. Group III sorghums are bird resistant and contain vanillin-reactive procyanidins that are soluble in methanol. Group II sorghums are intermediate in bird resistance and contain vanillin-reactive procyanidins that are soluble in acidic methanol. In plant materials that contain proanthocyanidins, a large portion can be insoluble in neutral–aqueous–organic solvents (Bate-Smith, 1975). Insoluble proanthocyanidins are present in neutral-detergent fiber (NDF) and are negatively correlated with NDF digestibility (Reed, 1986). This study also determines relationships among soluble phenolics, soluble and insoluble procyanidins, fiber, and fiber digestibility in sorghum grain.

International Livestock Centre for Africa, Addis Ababa, Ethiopia.

MATERIALS AND METHODS

Samples and Sample Preparation. Seventeen bird resistant (BR) and seven non bird resistant (NBR) sorghum varieties were grown at Debre Zeit, Ethiopia (1900 m), in a complete randomized block design with four plots (six rows, 5 m long, 75 m between rows) for each variety. Characteristics of the sorghum varieties have been reported elsewhere (Reed et al., 1987). Diammonium phosphate (100 kg ha^{-1}) was applied at planting, and urea (100 kg ha^{-1}) was applied at boot stage. Panicles from four post-ripe plants from each plot were harvested and combined, and the grain was separated by hand and ground

Table I. Paired Comparisons of Methods for Determining the Content of Neutral-Detergent Fiber (NDF) and Insoluble Procyanidins in NDF from Bird Resistant and Non Bird Resistant Sorghum Grain

	amylase method		urea/amylase method		difference	
	mean	std error	mean	std error	mean	std error
overall (n = 24)						
NDF (% OM)	14.2	0.8	12.6	0.8	1.6**	0.2
insol procyanidins (A_{550})	0.546	0.111	0.439	0.110	0.107**	0.023
bird resistant var (n = 17)						
NDF (% OM)	14.9	0.8	12.9	0.8	2.0**	0.2
insol procyanidins (A_{550})	0.747	0.128	0.596	0.138	0.151**	0.025
non bird resistant var (n = 7)						
NDF (% OM)	12.4	0.8	12.0	0.8	0.4	0.2
insol procyanidins (A_{550})	0.058	0.033	0.058	0.026	0.000	0.019

[a] Key: **, $p < 0.01$.

to pass a 1-mm screen in a hammer mill.

Analysis of Fiber, Fiber Digestibility, and Lignin. Neutral-detergent fiber (NDF) was determined by two methods: a procedure that uses heat-stable amylase (Catalog No. 1278, Sigma Chemical Co., St. Louis, MO) (Robertson and Van Soest, 1980) and a procedure that uses urea (8 M) and heat-stable amylase (Van Soest and Robertson, 1985). In vitro digestibility of NDF at 48 h, acid-detergent fiber (ADF), and acid-detergent lignin were determined by the methods of Goering and Van Soest (1970).

Analysis of Soluble Procyanidins and Soluble Phenolics. The vanillin/HCl method was used to determine soluble procyanidins (Guiragossian et al., 1977). A method based on precipitation with ytterbium acetate was used to determine soluble phenolics (Yb-ppt) (Reed et al., 1985). The method was modified by increasing sample weight (300 mg) and extracting with aqueous acetone (3 mL, 7:3 (v/v) acetone/H_2O) in test tubes (15 mL) placed in an ultrasonic water bath (10 °C, 1 min) five times. Soluble phenolics were isolated by precipitation with ytterbium and resolution with oxalic acid in order to determine their effect on degradability of NDF from grass by fungal cellulase (Reed et al., 1985).

Soluble red pigments were determined by extracting grain (300 mg) in aqueous ethanol (1:1 (v/v), 3 mL) in test tubes placed in an ultrasonic water bath (10 °C, 1 min) five times. The solution was filtered through Gooch crucibles (Pyrex, coarse porosity) and diluted to 100 mL with aqueous ethanol and absorbance measured at 550 nm.

Analysis of Insoluble Procyanidins in Neutral-Detergent Fiber. Two methods were used to determine insoluble procyanidins in NDF. Grain was extracted with aqueous acetone followed by preparation of NDF by either the amylase procedure or the urea/amylase procedure. NDF was heated (95 °C, 1 h) in butanol containing concentrated HCl (5 mL, 95:5 (v/v) butanol/HCl). Absorbance of the solution was measured at 550 nm (Reed et al., 1982). All analyses were conducted in duplicate. Colorimetric determinations were expressed in absorbance units. Gravimetric determinations were expressed as percentages of organic matter (% OM).

Statistical Analysis. Differences between BR and NBR varieties were tested for significance by the Student's t-test. Differences between methods for determining NDF and insoluble procyanidins in NDF were tested by the Student's t-test for paired comparisons. Linear correlation coefficients were used to describe relationships among phenolics, fiber, fiber digestibility, and degradability of NDF by cellulase. One BR variety was a group II type sorghum. This variety was excluded from comparisons between BR and NBR varieties because it was an outlier in phenolic composition but was included in the paired comparisons for the differences between methods for determining NDF and insoluble procyanidins.

Table II. Differences between Bird Resistant and Non Bird Resistant Sorghum Grain in Content of Neutral-Detergent Fiber (NDF) as Determined by the Amylase or Urea/Amylase Methods and Acid-Detergent Fiber (ADF)

	bird resistant var (n = 16)		non bird resistant var (n = 7)		difference	
	mean	std error	mean	std error	mean[a]	std error
NDF (% OM)						
amylase	14.8	0.9	12.4	0.8	2.4	1.5
urea/amylase	12.8	0.8	12.0	0.8	0.8	1.4
ADF (% OM)	7.5	0.4	6.3	0.4	1.2*	0.7

[a] Key: *, $p < 0.05$.

RESULTS AND DISCUSSION

Comparison of Methods for Determining Neutral-Detergent Fiber and Insoluble Procyanidins in Neutral-Detergent Fiber. The content of NDF prepared by the amylase procedure was significantly higher than the content of NDF prepared by the urea/amylase procedure (Table I). Insoluble procyanidins in NDF were significantly higher for the amylase procedure. The difference between BR and NBR varieties in NDF as prepared by either method was not significant (Table II), although the difference between varieties was greater for the amylase procedure. The urea/amylase procedure may remove more procyanidins, resulting in lower NDF. The difference between NDF methods should be greater in BR varieties that contain procyanidins. When the paired comparisons were conducted with BR varieties, the difference between methods for both NDF and insoluble procyanidins in NDF was significant, and when conducted with NBR varieties the difference between methods was not significant (Table I).

ADF in BR varieties was significantly higher than in NBR varieties (Table II). The organic fraction of ADF contains lignocellulose and other extracellular components such as cutin. The primary purpose for preparing ADF is for the determination of lignin (Van Soest, 1982). However, a small amount of anthocyanidin and a large amount of brown "phlobaphene-like" polymer is formed when proanthocyanidins are heated in aqueous acids (Swain and Hillis, 1959). Although the acid-detergent solution turned red while refluxing BR varieties, some procyanidins polymerized when refluxed in acid detergent (Reed et al., 1982). These procyanidins would be recovered in ADF and lignin, leading to higher content in BR varieties.

Relationships among Phenolics. The content of lignin, soluble and insoluble procyanidins, Yb-ppt, and soluble red pigments in BR varieties were significantly higher

Table III. Differences between Bird Resistant and Non Bird Resistant Sorghum Grain in Content of Lignin, Insoluble Procyanidins as Determined by the Amylase or the Urea/Amylase Procedures, Soluble Procyanidins, Soluble Phenolics Precipitated with Ytterbium Acetate (Yb-ppt), and Soluble Red Pigments

	bird resistant var (n = 16)		non bird resistant var (n = 7)		difference	
	mean	std error	mean	std error	mean[a]	std error
lignin (% OM)	2.2	0.2	1.2	0.2	1.0**	0.4
insol procyanidins (A_{550})						
amylase	0.625	0.039	0.058	0.033	0.566**	0.064
urea/amylase	0.464	0.046	0.058	0.026	0.406**	0.073
sol procyanindins (A_{500})	0.688	0.037	0.015	0.011	0.673**	0.057
Yb-ppt (% OM)	9.9	0.2	5.7	0.2	4.2**	0.4
sol red pigments (A_{550})	0.032	0.004	0.006	0.001	0.026**	0.006

[a] Key: **, $p < 0.01$.

Table IV. Linear Correlation Coefficients among Lignin, Insoluble Procyanidins as Determined by the Amylase or Urea/Amylase Procedures, Soluble Procyanidins, Soluble Phenolics Precipitated with Ytterbium Acetate (Yb-ppt), and Soluble Red Pigments in Bird Resistant and Non Bird Resistant Sorghum Grain

	lignin[a]	insol procyanidins: amylase	insol procyanidins: urea/amylase	sol procyanidins	Yb-ppt
overall correlations (n = 23)					
insol procyanidins					
amylase	0.683**				
urea/amylase	0.747**	0.932**			
sol procyanidins	0.310	0.744**	0.571**		
Yb-ppt	0.343	0.712**	0.547**	0.942**	
sol red pigments	0.711**	0.779**	0.686**	0.604**	0.617**
bird resistant grain (n = 16)					
insol procyanidins					
amylase	0.599*				
urea/amylase	0.644**	0.848**			
sol procyanidins	−0.613**	−0.588*	−0.696**		
Yb-ppt	−0.405	−0.582*	−0.646**	0.646**	
sol red pigments	0.596*	0.494*	0.326	−0.190	−0.093

[a] Key: *, $p < 0.05$; **, $p < 0.01$.

than in NBR varieties (Table III). When both BR and NBR varieties were included in the correlation analysis, lignin was significantly and positively correlated with insoluble procyanidins and soluble red pigments but nonsignificantly correlated with soluble procyanidins and Yb-ppt (Table IV). Insoluble procyanidins were significantly and positively correlated with soluble procyanidins, Yb-ppt, and soluble red pigments. Correlations among soluble procyanidins, Yb-ppt, and soluble red pigments were significant and positive.

The significant correlation between lignin and insoluble procyanidins indicates that procyanidins may contribute to the lignin fraction. The high positive correlation between soluble procyanidins and Yb-ppt indicates that ytterbium precipitates vanillin-reactive phenolics.

Significant linear correlations among phenolics could result from the large differences between BR varieties that are high in phenolics and NBR varieties that are low in phenolics. When correlation analysis was conducted with BR varieties only, lignin was positively and significantly correlated with insoluble procyanidins, Yb-ppt was significantly and positively correlated with soluble procyanidins, but lignin and insoluble procyanidins were significantly and negatively correlated with soluble procyanidins and Yb-ppt (Table IV). Within the BR varieties, as the quantity of vanillin reactive-soluble procyanidins and Yb-ppt decreased, the quantity of insoluble procyanidins and lignin increased.

The most extreme case for this inverse relationship was in the group II variety, not included in the correlation analysis. The soluble procyanidins and Yb-ppt in this variety were similar to NBR varieties, 0.030 absorbance unit and 4.7% OM, respectively. However, the amount of insoluble procyanidins was much higher than in the BR varieties; 5 mg of NDF from the group II variety gave twice the absorbance at 550 nm than 10 mg of NDF from the group III BR varieties. This variety also had the highest lignin and ADF content, 3.6% and 10.6% OM, respectively.

Table V. Differences between Bird Resistant and Non Bird Resistant Sorghum Grain in Residual Amount of Neutral-Detergent Fiber (RNDF) and Digestibility of NDF (DNDF) after in Vitro Fermentation for 48 h and Degradability of NDF from Grass by Fungal Cellulase after Addition of Soluble Phenolics

	bird resistant grain (n = 16)		non bird resistant grain (n = 7)		difference	
	mean	std error	mean	std error	mean[a]	std error
RNDF (% OM)	6.7	0.6	2.5	0.3	4.2**	0.9
DNDF (%)	55.3	1.7	79.9	2.1	−24.6**	2.9
NDF degrad (%)	45.5	0.4	52.3	0.3	−6.9**	0.7

[a] Key: **, $p < 0.01$.

Phenolics, Fiber Digestibility, and Inhibition of Cellulase. The residual amount of NDF (RNDF) after in vitro fermentation for 48 h in BR varieties was significantly higher than in NBR varieties (Table V). The digestibility of NDF (DNDF) in BR varieties was significantly lower than in NBR varieties. All phenolics were significantly and positively correlated with RNDF and significantly and negatively correlated with DNDF (Table VI). Lignin and insoluble procyanidins had higher correlations with RNDF than soluble procyanidins and Yb-

Table VI. Correlation of Phenolics with Residual Neutral-Detergent Fiber (NDF), Digestibility of NDF (DNDF), and Degradability of NDF from Grass by Fungal Cellulase

	RNDF[a]	DNDF	NDF degrad
lignin	0.881**	−0.705**	NA[b]
insol procyanidins			
amylase	0.784**	−0.851**	NA
urea/amylase	0.815**	−0.799**	NA
sol procyanidins	0.559**	−0.746**	−0.932**
Yb-ppt	0.620**	−0.760**	−0.893**
sol red pigments	0.734**	−0.627**	NA

[a] Key: **, $p < 0.01$. [b] Not applicable, only soluble phenolics added to the cellulase system.

ppt. Lignin and insoluble procyanidins may be covalently bound to cell wall carbohydrates, which may inhibit microbial digestion of these carbohydrates (Gupta and Haslam, 1980).

Soluble phenolics from BR varieties that were isolated by the ytterbium method significantly depressed degradability of NDF by cellulase when compared to soluble phenolics from NBR varieties (Table V). Degradability of NDF in the control (no phenolics added) was 52.5%. Concentrations of soluble procyanidins and Yb-ppt were significantly and negatively correlated with degradability of NDF (Table VI).

Soluble phenolics from BR varieties inhibit cellulase. These phenolics can be isolated by precipitation with ytterbium and resolution with oxalic acid (Reed et al., 1985). The gravimetric method based on precipitation with ytterbium or the measurement of procyanidins by vanillin-/HCl give a good indication of the amount of soluble, inhibitory phenolics in sorghum grain.

CONCLUSION

These results indicate that the ytterbium method for determination and isolation of soluble phenolics is appropriate for use in sorghum grain because the quantity of phenolics precipitated is highly correlated with their reactivity to vanillin/HCl, and the phenolics so isolated from BR varieties inhibit cellulase activity.

However, not all inhibitory phenolics are soluble. Insoluble procyanidins may increase the ADF and lignin fractions and decrease digestibility of NDF. Concentrations of soluble and insoluble procyanidins are inversely related in BR varieties. This inverse relationship indicates that measurement of Yb-ppt or soluble procyanidins may give an unrealistic estimate of the total amount of inhibitory phenolics present in BR varieties. Measurements of insoluble procyanidins and/or lignin should be included in studies on the nutritive value of sorghum grain.

ABBREVIATIONS USED

ADF, acid-detergent fiber; BR, bird resistant; DNDF, digestibility of neutral-detergent fiber; NBR, non bird resistant; NDF, neutral-detergent fiber; OM, organic matter; RNDF, residual amount of neutral-detergent fiber after in vitro fermentation; Yb-ppt, amount of soluble phenolics precipitated by ytterbium.

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