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Composition and in vitro gas production of whole stems and cell walls of different genotypes of pearl millet and sorghum

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Abstract

The top three senescent internodes of six genotypes of pearl millet (ICMV 155 Nor 155, ICMV 155 bmr, ICMV 221, ICMH 356, ICMH 94410, NCD 2) and sorghum (CSV 15, CSH 9, local yellow, ICSV 93046, ICSV 89057, ICSV 112) were used to asses the relationship between cell walls and wall components and in vitro gas production. Walls, (nitrogen) N, total soluble sugar contents and gas production in stems of both sorghum and pearl millet were significantly different between genotypes. In pearl millet stems, lignin and esterified *p*-coumaric acid (*p*CA) were significantly different between genotypes. In sorghum, N, lignin and phenolic acids—except esterified *p*CA were significantly different between genotypes. Except at 12 h for sorghum, gas production of walls was significantly different between genotypes and ranked genotypes differently than gas of stems.

Soluble sugars in the stems of both pearl millet and sorghum were positively correlated to gas production at 12, 24, and 36 h, and vice versa for cell walls. In pearl millet, phenolic acids were not significantly correlated with either gas production of whole stems or that of walls, with the exception of etherified *p*CA of walls. On the other hand, in sorghum, etherified *p*CA of stems and walls were negatively correlated with gas at 72 and 96 h. The significant differences of gas production between genotypes, different ranking of genotypes with the progression of the fermentation, and the gas production differences between whole stems and walls indicate the differential effect of soluble sugars and of cell wall type on the digestibility of whole stems. This study points out the need to look in greater details at the relationship and the interactions between the effects of soluble and structural carbohydrates in stovers and the genetic base that determines it. Such studies could identify the most adequate combination of traits useful in crop improvement programs taking into account feed value of stover of dual-purpose crops.

Keywords: Sorghum; Phenolic acid; Lignin; Millet; Gas production

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1. Introduction

In developing countries crop residues are the main source of carbon and nitrogen (N) for ruminants. The composition and physical arrangement of the cell wall of these roughages (stovers, straws, bagasse, etc.) affects the relative accessibility of wall polysaccharides to rumen microbial enzymes and thus control their digestibility and utilization. Lignin, the second largest component of walls (by weight) after cellulose, is known to interfere with the digestion of cell wall polysaccharides. In grasses, lignin is linked to wall polysaccharides either directly or through other molecules in the wall such as phenolic acids (Iiyama et al., 1994; Lam et al., 1990c). Phenolic acids (*p*-coumaric acid (*p*CA) and ferulic acid (FA)) can form linkages with polysaccharides and ether-linkages with lignin (Lam et al., 1992a, 1994a). There is evidence that the degree of phenolic acid cross-linking is negatively correlated to IVDMD. The linkages are known to interfere with the degradation of wall polysaccharides (Lam et al., 1993).

In grasses, the make up of wall polysaccharides mainly consist of cellulose (60%) and arabinoxylans (25%). In addition, lignin deposited on and between the wall polymers provides a hydrophobic environment that reduces the accessibility of hydrophilic molecules and hence, the degradation of the cell wall sugars (Lam et al., 1993). As consumption of cell walls becomes a limitation with increasing animal production potential, the balance of soluble and structural carbohydrates in forages fed to ruminants also becomes limited (Mertens, 1995).

Variations in these parameters amongst different genotypes could be used to differentiate between quality in pearl millet and sorghum and therefore, be a useful tool for breeders in crop improvement programmes.

The objective of this study was to estimate the content of cell walls, total soluble sugars, and N in whole stems and lignin, different forms of FA, and *p*CA in wall polymers of stovers of contrasting genotypes of pearl millet and sorghum and to assess their relationship with digestibility as measured by the in vitro gas production method.

2. Materials and methods

2.1. Sorghum and pearl millet genotypes

The top three internodes of 10 plants of four field replicates of six mature (senescent) genotypes of pearl millet (ICMV 155 Nor 155, ICMV 155 bmr, ICMV 221, ICMH 356, ICMH 94410, NCD 2) and sorghum (CSV 15, CSH 9, local yellow, ICSV 93046, ICSV 89057, ICSV 112) dried at 65 °C overnight were ground in a Wiley mill to pass a 1 mm mesh. Dry matter of ground samples and extracted walls were determined according to AOAC (1980) procedure. The main characteristics of sorghum and pearl millet genotypes are presented in Table 1. Sorghum and pearl millet genotypes were grown at the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), India, during the main rainy season in 1998 in broad beds on black cotton soil and red soil, respectively on which 50 kg of diammonium phosphate (DAP; 18% N; 46% P) per hectare had been applied.

Table 1
Description of pearl millet and sorghum genotypes characteristics

Genotype	Characteristics
Pearl millet	
ICMH 356	Hybrid developed at ICRISAT and released in 1993; grain yield up to 2.6 t ha^{-1}; it matures at 75–80 days with plant height of 160–200 cm; resistant to Downy mildew
ICMV 155	Dual-purpose open pollinated variety released in 1991; it matures at 80–100 days with plants height of 180–240 cm; it has thick stems, many leaves and 2–4 tillers; it is resistant to Downy mildew; it yielded 12% more grain and 9% more fodder than WC C75
ICMV 155 (bmr)	Plant type similar to ICMV 155 described. Produced by backcrossing a bmr line received from USA to ICMV 155; the plants are distinct with the brownish pigmentation on stems and leaves; grain and fodder yields are similar to that of ICMV 155
NC D2	It is a dwarf population derived from Nigerian composite (NC-tall); grain yield up to 2.9 t ha^{-1} with plant height of 120 cm; it flowers at 50–52 days; highly resistant to Downy mildew
ICMH 94410	Grian type, early maturing, dwarf hybrid developed at ICRISAT
ICMV 221	High grain yielding bold seeded, open pollinated variety released in 1993; it flowers in 38–50 days, matures in 70–80 days and performs well under terminal drought conditions; plants are thick stemmed of 140–200 cm height; it is resistant to Downy mildew
Sorghum	
Local yellow	Local landrace usually cultivated in main rainy season; it flowers in about 70 days with average plant height of 170 cm
CSH 9	Hybrid released by the Indian program in the 1970s for cultivation in the rainy season; grain yields up to 3.7 t ha^{-1} and fodder yield up to 13 t ha^{-1}; it flowers in about 71 days with average plant height at maturity of 200 cm
CSV 15	Dual purpose variety developed by the Indian program; when cultivated in the rainy season it has grain yield up to 3.3 t ha^{-1} and fodder yield is 15 t ha^{-1}; it flowers in 70 days, and matures in 106 days; plant height up to 250 cm; moderately resistant to shoot fly, stem borer, and grain mold
ICSV 112	Variety developed at ICRISAT widely adapted around the globe (India, Malawi, Mexico, Nicaragua, Zimbabwe, etc); grain yield up to 3.4 t ha^{-1}, and fodder yield is 11.4 t ha^{-1}; it matures at 110–120 days with plant height of 150–180 cm; it is a photoperiod-insensitive, rainy season variety; it grows well also during the postrainy season; it is moderately resistant to shoot fly and stem borer
ICSV 89057	Line developed at ICRISAT through backcrossing and selection involving parents like ICSV 112 and ICSV 197; it flowers in 62 days with plant height at maturity up to 260 cm; grain yield up to 4.8 t ha^{-1}; it can be used as a fodder line; it is tolerant to midge and head bug
ICSV 93046	Sweet stalk line developed at ICRISAT; during rainy season it flowers in 62 days and grows to a height of 220 cm; it is comparatively tolerant to stem borer and shoot fly

2.2. Walls,wall lignin and phenolic acids

Walls were obtained by extracting soluble plant components (sugars, proteins, etc.) from ground samples according to the method of Lam et al. (1992a). Lignin content was determined using the method developed by Iiyama and Wallis (1990) (acetyl bromide method). Esterified phenolic acids were extracted from cell wall samples according to Lam et al. (1990c). A sample of 100 mg was treated with 1 M NaOH overnight at room temperature with *m*-coumaric acid added as an internal standard. The solution was then

acidified to pH 1.0 and extracted successively with dichloromethane and with diethyl ether. The solvent was removed by evaporation and the sample was trimethylsilylated by adding BSTFA and keeping at 100 °C for 5 min. Total phenolic acids were extracted using the method of Iiyama et al. (1990) and Lam et al. (1994b). A sample of 30 mg was treated with 4 M NaOH for 2 h at 170 °C with *m*-coumaric acid added as an internal standard. The solution was then acidified to pH 1.0 and extracted with dichloromethane and with diethyl ether. The solvent was removed by vacuum evaporation and the sample was trimethylsilylated by adding BSTFA and keeping at 100 °C for 5 min.

Esterified and total concentrations of *p*CA and FA were determined by gas chromatography (GC) using a Unicam 610, UK a capillary column Neutrabond-1. Starting column temperature was 180 °C for 5 min, increasing 5 °C every minute up to a maximum of 280 °C. Maximum temperature was maintained for 5 min. The injector and detector were maintained at 280 °C. The injection sample size was 1 μl and the split ratio was 1/30. The amount of etherified FA and *p*CA were calculated by subtracting the content of respective esterified forms from the total acid content.

2.3. Total soluble sugars and nitrogen

Soluble sugars in whole stems were determined according to the method of Dubois et al. (1956). Nitrogen in whole stems and walls was determined using a Technicon autoanalyser (Industrial method, 1972).

2.4. In vitro gas production

Total gas production of stems and extracted walls was determined according to Menke et al. (1979), as modified by Osuji et al. (1993). Blanks and 200 mg of ground samples were incubated in triplicates with 30 ml of a mixture of strained rumen fluid and buffer containing sodium hydrogen carbonate. The rumen fluid was obtained from four bullocks (average live weight = 388 kg; standard deviation (S.D.) = 40.3), before the morning feeding, and adapted to a diet of sorghum residues for 3 weeks. Cumulative fermentation gas was measured at 12, 24, 36, 48, 72 and 96 h.

2.5. Statistical analysis

Chemical parameters and gas production were analyzed with PROC GLM of SAS (SAS, 1989) with genotype as the source of variation. Pearson's correlation was carried out between chemical parameters and gas values.

3. Results

3.1. Walls, N, soluble sugar content and total gas

Walls, N and total soluble sugar contents in stems of both sorghum and pearl millet were significantly different between genotypes (Table 2). With the exception of yellow sorghum,

Table 2
Cell walls, nitrogen, total soluble sugars and cumulative gas production (in ml/200 mg dry matter) at 12, 24, 36, 48, 72 and 96 h of incubation of stems of millet and sorghum genotypes

Genotype	Dry matter (%)	Cell wall (dry matter (%))	N (dry matter (%))	Total soluble sugars	Gas 12	Gas 24	Gas 36	Gas 48	Gas 72	Gas 96
Millet										
ICMV 155 Nor	97.33	77.6	0.27	3.86	6.08	11.02	17.01	21.24	29.91	37.33
ICMV 155 bmr	93.98	80.1	0.29	1.57	3.45	9.35	14.76	19.72	27.26	32.45
ICMV 221	92.59	73.8	0.27	10.74	9.17	14.65	18.88	22.92	28.37	32.67
ICMH 356	94.71	81.1	0.29	1.31	4.16	8.28	14.55	19.11	24.95	31.26
ICMH 94410	93.89	80.3	0.39	1.26	4.20	8.76	15.00	19.16	25.37	31.43
NCD2	93.68	83.4	0.26	0.86	4.08	9.37	15.72	22.65	31.59	35.99
SE		1.0	0.01	0.15	0.23	0.25	0.30	0.31	0.25	0.16
F test		***	***	***	***	***	***	***	***	***
Sorghum										
CSV 15	94.25	79.4	0.16	5.23	7.20	12.23	17.53	23.94	37.82	45.24
CSH 9	95.61	82.6	0.15	1.36	4.22	9.50	16.73	26.23	42.58	58.86
Local yellow	94.66	84.0	0.13	8.20	6.02	10.15	16.13	21.23	29.89	36.26
ICSV 93046	91.74	66.6	0.14	22.62	14.39	19.43	25.29	30.33	38.05	43.13
ICSV 89057	93.55	78.2	0.12	5.54	7.66	12.78	18.88	26.50	38.21	44.71
ICSV 112	94.22	82.4	0.17	2.34	5.13	9.25	16.42	21.90	32.89	42.26
SE		0.5	0.00	0.34	0.21	0.20	0.28	0.35	0.28	0.29
F test		***	***	***	***	***	***	***	***	***

N: nitrogen; gases 12, 24, 36, 48, 72, 96: gas production from in vitro fermentation at 12, 24, 36, 48, 72 and 96 h of incubation, respectively.
*** Significant at $P < 0.001$.

higher concentrations of walls were associated with lower soluble sugar contents and vice versa in both pearl millet and sorghum. Gas production in both pearl millet and sorghum stems significantly differed between genotypes at all hours of fermentation. In ICMV 221 and ICMV 155 pearl millet genotypes, higher gas values, especially during the first 24 h of fermentation, were associated with higher content of soluble sugars. On the other hand, higher gas values of stems of sorghum genotypes were not always associated with higher soluble sugar contents, except for genotype ICSV 93046. Differences in gas production between genotypes of both sorghum and millet were not consistent throughout the fermentation period. After 24–36 h the ranking of genotypes changed possibly due to a smaller effect of the soluble sugars and an increasing importance of the type of cell wall available for fermentation in different genotypes.

3.2. Lignin, N, esterified and etherified p-coumaric acid (pCA), ferulic acid (FA) and total gas production of walls

Lignin (17.1–20.7%), N (0.20–0.36%) and esterified *p*CA (0.44–1.05%) contents in pearl millet stems were significantly different between genotypes (Table 3). In sorghum, N (0.10–0.14%) and all phenolic acid components, except esterified *p*CA, and lignin (19.4–21.9%), were significantly different. In both sorghum and pearl millet more than 80% of the N content of the stems was present in the wall preparation. Gas production of walls was significantly different between genotypes except at 12 h for sorghum genotypes (Table 3). In pearl millet, gas production from cell wall extracts was lower than that from whole stems up to 24 h of incubation. However, at 36 h and following hours of fermentation, gas production from walls was greater than that of whole stems except for two genotypes (ICMV 155 and NCD2), thus changing the ranking of genotypes relative to gas production. On the other hand, in all sorghum genotypes, cell wall gas production at all hours was lower than that from whole stems. Differences in the gas values for stem and walls at all hours were highest in ICSV 93046. However, at 96 h the greater difference was for CSH 9. Interestingly, ICSV 93046 and CSH 9 contained the highest and lowest amount of soluble sugars in the stem.

3.3. Correlations

Soluble sugars in the stems of both pearl millet and sorghum genotypes were positively correlated to gas production at 12, 24, 36 and 48 h of incubation (Table 4). However, this correlation was significant only up to 36 h. On the other hand, there was a significant negative correlation between wall contents and gas production up to 36 h for millet and 48 h for sorghum but not thereafter. This correlation seemed to be stronger in sorghum than in pearl millet. The apparent negative correlation between N and gas production for whole stems and wall in both pearl millet and sorghum was not significant.

The negative correlation between lignin and gas production of whole stems was significant up to 12 h in pearl millet but up to 36 h in sorghum. In pearl millet, different forms of phenolic acids were not significantly correlated with either gas production of whole stems or that of walls, with the exception of etherified *p*CA of walls, positively correlated with gas at 48 h. In sorghum, etherified *p*CA of stems and walls were negatively

Table 3
Lignin, nitrogen, esterified and etherified *p*CA, FA and total gases (in ml/200 mg cell wall) at 12, 24, 36, 48 and 72 h of incubation of pearl millet and sorghum genotypes

Genotype	Lignin	N	*p*CA (% cell wall)		FA (% cell wall)		Gas 12	Gas 24	Gas 36	Gas 48	Gas 72	Gas 96
			Est	Eth	Est	Eth						
Millet												
ICMV 155 Nor	18.7	0.20	0.82	0.68	0.33	1.53	2.60	7.44	13.76	19.14	30.28	36.78
ICMV 155 bmr	19.1	0.26	0.44	0.38	0.27	1.24	2.24	5.73	10.78	16.19	27.32	34.64
ICMV 221	17.1	0.23	1.05	0.58	0.36	1.59	3.53	9.76	15.98	19.56	29.70	35.27
ICMH 356	19.6	0.27	1.01	0.67	0.23	1.46	2.41	7.06	13.10	19.53	27.96	34.53
ICMH 94410	18.3	0.36	0.82	0.91	0.24	1.38	2.90	8.64	15.97	22.33	31.25	38.90
NCD 2	20.7	0.22	0.80	0.61	0.19	1.16	2.73	8.95	14.46	19.92	27.95	33.96
SE	0.54	0.01	0.04	0.14	0.03	0.14	0.22	0.30	0.29	0.28	0.32	0.21
F test	**	***	***	NS	NS	NS	*	***	***	***	***	***
Sorghum												
CSV 15	16.6	0.14	1.05	1.06	0.38	0.33	2.17	5.98	11.74	17.55	30.27	39.76
CSH 9	17.5	0.12	1.37	0.77	0.12	0.87	2.39	7.93	13.78	20.09	35.05	46.50
Local yellow	16.6	0.10	1.03	1.74	0.36	1.06	2.87	8.11	13.41	19.73	30.17	36.50
ICSV 93046	13.9	0.13	1.33	1.92	0.39	1.16	2.73	6.99	12.67	18.56	27.81	32.73
1CSV 89057	15.20	0.11	1.02	1.32	0.25	1.36	2.81	6.44	13.54	17.34	30.10	40.65
ICSV 112	18.1	0.14	1.18	1.30	0.27	1.15	2.68	7.37	13.41	19.59	32.75	37.86
SE	0.79	0.00	0.07	0.16	0.05	0.16	0.18	0.18	0.18	0.24	0.28	0.28
F test	*	***	NS	***	*	*	NS	***	***	***	***	***

N: nitrogen; *p*CA: *p*-coumaric acid; FA: ferulic acid; Est: esterified; Eth: etherified; gases 12, 24, 36, 48, 72, 96: gas production from in vitro fermentation at 12, 24, 36, 48, 72 and 96 h of incubation, respectively NS: Not significant.

* Significant at $P < 0.05$.

** Significant at $P < 0.01$.

*** Significant at $P < 0.001$.

Table 4
Pearson's correlation coefficients of gas production of whole stem and cell walls with soluble sugars, cell walls and lignin in pearl millet and sorghum ($n = 6$)

	Whole stem						Cell wall					
	Gas 12	Gas 24	Gas 36	Gas 48	Gas 72	Gas 96	Gas 12	Gas 24	Gas 36	Gas 48	Gas 72	Gas 96
Millet												
Soluble sugars	0.98***	0..98***	0.93**	0.61	0.15	0.01	–	–	–	–	–	
Cell wall	−0.91*	−0.90*	−0.84*	−0.36	0.02	0.03						
Nitrogen	−0.35	−0.42	−0.45	−0.68	−0.69	−0.60	−0.03	−0.02	0.22	0.52	0.35	0.61
Lignin	−0.82*	−0.79	−0.72	−0.18	0.21	0.20	−0.66	−0.31	−0.43	−0.13	−0.62	−0.50
Esterified FA	0.76	0.79	0.74	0.28	0.06	0.14	0.47	0.12	0.14	−0.24	−0.38	0.19
Etherified FA	0.57	0.36	0.41	−0.11	−0.37	−0.11	0.50	0.26	0.40	−0.20	0.55	0.33
Esterified *p*CA	0.45	0.22	0.33	0.21	−0.12	0.10	0.63	0.67	0.70	0.60	0.37	0.06
Etherified *p*CA	−0.13	−0.33	−0.19	−0.29	−0.29	−0.10	0.30	0.47	0.72	0.95**	0.79	0.78
Sorghum												
Soluble sugars	0.96**	0.93**	0.92**	0.65	−0.05	−0.33	–	–	–	–	–	
Cell wall	−0.97***	−0.99***	0.99***	−0.86*	−0.30	−0.01	–	–	–	–	–	
Nitrogen	−0.39	−0.41	−0.37	−0.41	−0.06	0.19	−0.54	−0.46	−0.51	−0.51	0.06	−0.11
Lignin	−0.91*	−0.92**	−0.89**	−0.77*	−0.19	0.16	−0.48	0.07	−0.17	0.40	0.43	0.05
Esterified FA	0.37	0.29	0.14	−0.29	−0.67	−0.08	0.12	−0.39	−0.73	−0.42	−0.86*	−0.85*
Etherified FA	−0.04	−0.08	0.01	−0.02	−0.31	−0.29	0.91*	0.33	0.71	0.13	−0.15	−0.27
Esterified *p*CA	−0.45	−0.41	−0.27	0.01	0.40	0.63	−0.20	0.36	0.19	0.50	0.33	0.14
Etherified *p*CA	0.43	0.33	0.28	−0.15	−0.79	−0.93**	0.71	0.10	−0.10	−0.07	−0.81*	−0.93**

* Significant at $P < 0.05$.
** Significant at $P < 0.01$.
*** Significant at $P < 0.001$.

correlated with gas at 96, 72 and 96 h, respectively. Etherified FA of walls were positively correlated to gas at 12 h.

4. Discussion

4.1. N

The N content of stems of pearl millet (0.29%) was about twice that of sorghum (0.14%). Walls contained more than 80% of the total N in stems, suggesting that most protein in the stem was present as structural protein in walls. These structural proteins in the wall matrix may play a critical role in cross-linking components, particularly in primary walls and they may be more resistant to microbial degradation in the rumen (Hatfield et al., 1999). Although, this statement supports the negative correlation values of N with gas production, it is not clear whether such low N content could have any significant effect on gas production. Restricted accessibility to polysaccharides is likely to be the explanation, not N per se. On the other hand, at such low N concentrations even small changes due to genotype could affect stover degradation in the rumen and feed utilization by the animal.

4.2. Soluble sugars

The total soluble sugars in the stem internodes of sorghum were higher than those in pearl millet (7.6 versus 3.3%). Additionally, in the initial phase of incubation, the average gas production of the whole stem of sorghum tended to be higher than that of pearl millet (7.4, 12.2 and 18.5 versus 5.2, 10.2 and 16.0, respectively). Highly degradable soluble sugars are easily accessible to degradative enzymes, and had an extensive effect on whole stem degradation, especially during the early hours of fermentation. With the progression of the fermentation time, the effects of these sugars on gas production decreased but there were notable differences between genotypes. The effect of soluble sugars on the fermentation in the gas test is expected and could involve a different set of organisms to the ones that are fermenting cellulose. It is possible that the presence of these sugars in some way also assists the digestion of the cell wall polysaccharides. For example, the gas production at 48 h ICMV221 and NCD2 were similar (Table 2), but the differences in gas production between walls and stems for ICMV221 at 12 and 24 h were larger than those observed for the stems and walls of NCD2 (Tables 2 and 3). Total soluble sugars in ICMV221 were higher than those in NCD2, accounting for greater stem digestion at 24 h. The very low contents of sugars in NCD2 were digested in the early hours of incubation and thus their effects were less in the later stages. On the other hand, these differences in gas production between whole stems and cell walls, during the early stages of fermentation, were much lower in genotypes such as ICMH 356 and ICMH 94410, which contained relatively lower amounts of total soluble sugars readily degradable in the initial phase of fermentation. The significant differences of gas production between genotypes and between whole stems and walls indicate the extent of the effect of soluble sugars on the digestibility of whole stems. This study points out the need to look in greater details at the relationship and at the interactions between the effects of soluble and structural

carbohydrates in stovers and the genetic base that determines it. This will allow a better understanding of the tradeoffs between the nutrients translocated to the grain during plant maturation, which will affect stover quality, and the genetic differences between genotypes of both sorghum and millet.

4.3. Lignin

Average lignin content (15%) of stem internodes of pearl millet was similar to that of sorghum genotypes (16.3%). However, these values were higher than those reported for sorghum (10.9% and 8.5%), pearl millet (10.8%), maize (11.0%) and wheat (14.0%) by Lam et al. (1996), and those of rice straw (8.5%) and phalaris (12.1%) reported by Iiyama and Wallis (1990) (Table 5). The negative correlation between lignin and gas production in whole stem of both sorghum and pearl millet agrees with a number of reports including that of Jung and Deetz (1993), who indicated that lignification of the walls limits microbial fermentation or enzymatic hydrolysis of forage wall polysaccharides. Partial delignification experiments conducted on pangola grass (*Digitaria decumbens*) showed that lignin was the major factor limiting total digestion of plant material in vitro (Ford, 1983). Other studies shows that lignin accounted for most of the variation in in vitro digestibility of stems (Buxton and Hornstein, 1986) and it was the primary predictor of cell wall degradability (Jung and Buxton, 1994). Ford (1983) has shown that the rate of cellulose degradation did not change when walls were delignified. If lignin-shielding were the only factor contributing to decreased cellulose degradation, then removal of lignin would

Table 5
Reported values of lignin, *p*CA and FA (esterified and etherified) concentration

Sample	Lignin (% cell walls)	*p*CA[a] (% cell walls)		FA (% cell walls)	
		Est	Eth	Est	Eth
Sorghum[a]					
Normal(S)	10.9	2.04	0.01	0.39	0.38
bmr6(S)	10.5	0.63	0.02	0.28	0.20
Normal(S)	8.5	1.54	0.00	0.33	0.32
bmr18(S)	6.2	0.62	0.07	0.39	0.24
This study		0.91	1.05	0.22	0.77
Pearl millet[a]					
Normal(S)	10.8	1.13	0.55	0.26	0.73
Bmr(S)	10.4	0.41	0.04	0.31	0.35
This study		0.61	0.51	0.21	1.10
Maize[a]					
Normal(S)	11.0	2.79	0.01	0.38	0.43
Bm3(S)	10.0	1.51	0.06	0.35	0.35
Wheat(1st internode)[b]	14.0				

[a] Lam et al., 1996.
[b] Lam et al., 1993.

potentially increase the rate of cellulose degradation. It seems likely that the hydrogen bonding between cellulose microfibrils could influence the rate of degradation, whereas lignin may only impact on the extent of degradation (Hatfield, 1989).

4.4. Phenolic acids

The level of esterified *p*CA of sorghum and millet stems found in this study were lower than those reported by Lam et al. (1996). On the other hand, etherified *p*CA of pearl millet and sorghum were similar and greater, respectively than those reported by Lam et al. (1996) (Table 5). The esterified FA observed in sorghum and pearl millet were lower while etherified FA were higher than those reported by Lam et al. (1996).

Wall FA did not show any correlation with gas production in either millet or sorghum except for etherified FA that correlated positively with gas from walls of sorghum at 12 h. This correlation could be a mathematical artifact. Lam et al. (1992b) reported that the relative amount of esterified FA decreased during maturation in walls of both wheat and phalaris internodes supporting the suggestion that etherification of esterified FA is occurring to produce FA ester–ether bridges between lignin and polysaccharides. The lower amount of esterified FA observed in this study compared to that observed by Lam et al. (1996) in sorghum and millet, would support this hypothesis. Poor correlation between ether-linked ferulates and cell wall degradability was also reported by Jung (1988) for maize internodes. These authors indicated that the current analytical method used for FA analysis might not yield all cross-linking structures between polysaccharides and lignin. In addition, they argue that during cell wall development the deposition pattern of ferulates may mask their relationship with wall degradation and that ground forage samples may not account for differences in cell wall composition of specific cellular tissues that may occur in different genotypes.

Esterified and etherified *p*CA were not significantly correlated with gas production at any time during the incubation in millet or sorghum with the exception of etherified *p*CA in millet walls at 48 h. It is likely that this acid is only linked to lignin in pearl millet and sorghum stems and would not affect degradation of wall polysaccharides.

This study suggests that when lignin content of senescent mature walls is high, the negative effect of phenolic acids on cell wall degradation is less apparent. These observations are supported by those of Van Soest (1993) who indicated that the effect of phenolic acids on wall degradation might be limited to less extensively lignified material. On the other hand, the effect of phenolic acid concentration on wall degradation was evident in wheat internodes during the later stages of maturation, when lignin was increased marginally but cross-linking between lignin and cell wall polysaccharides by FA increased substantially (Lam et al., 1993). Eraso and Hartley (1990) concluded that the negative effect of FA on wall degradability was greater when FA was in the dimeric form. They observed that total dimer to monomer ratio in sorghum stems was only 0.07. Only if these dimers link the polysaccharide chains, then the amounts may be sufficient to limit wall degradability. The studies by Lam et al. (1992b, 1990c, 1996) have been conducted with internodes at different maturity levels but of only one genotype, whereas, we compared mature senescent internodes of contrasting genotypes of millet and sorghum. In addition, linkages between lignin and wall polysaccharides during maturation of millet

and sorghum walls may differ substantially from those reported in the literature for wheat and phalaris.

The results from this study and those reported from the literature for sorghum and pearl millet suggest that phenolic acids may contribute differently to variations in gas production at different fermentation times depending on the concentration of lignin, their linkages with other cell wall polymers and the stage of plant maturity. The effect of phenolic acids is limited to walls with lower lignin content. Chemical association between polysaccharides and lignin through FA bridges may provide the best explanation for observed changes in dry matter digestibility with maturity (Lam et al., 1993). However, these associations may not be adequate for ranking feed quality of senescent crop residues of different genotypes.

The simple ranking of genotypes of millet and sorghum was different whether related to gas production of whole stems or of cell walls. This difference was more evident in sorghum. This indicates the effect of cell wall type as—a whole entity—on gas production especially during fermentation times greater than 48 h with clear differences between genotypes. These results suggest that total gas production from fermentation of whole stems may be a more adequate indicator of stem quality than gas of walls because the soluble components of the stem contribute significantly to the fermentation process, changing the ranking derived from cell walls fermentation only.

5. Conclusions

This study points out the difficulty of choosing one single trait, whether of the whole stem or of the cell wall, to be used as a parameter for improvement of digestibility. There seem to be an interaction between components of the wall and cell solubles such as soluble sugars and nitrogen resulting in differences in digestibility between genotypes. The other interesting issue emerging from this study is the relative importance to be given to parameters of the cell wall such as lignin and structural carbohydrates versus soluble sugars in cell contents. It appears that the importance of these traits in stover digestion and the differences between genotypes vary during the fermentation process. Therefore, it is suggested that soluble sugars, total cell walls, lignin and gas production be included into an evaluation index to evaluate genetic differences of stover quality of different cereals such as sorghum and millet.

While the genetic base of wall structure including lignin–polysaccharide and lignin–phenolic covalent bonds needs to be further elucidated, it will be important to consider the effects of genotypes on the changes in translocation of nutrients from the stover to the grain during plant maturation. The residual soluble components remaining in the stover and leaves after harvest could have as much impact on stover digestion and utilization as the amount and the composition of the walls.

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