Gene Action Governing Pearl Millet Stover Nitrogen and *in Vitro* Digestibility and Opportunities for Improvement

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ABSTRACT

Two pearl millet [*Pennisetum glaucum* (L.) R. Br.] stover fodder traits, nitrogen content (N) and *in vitro* digestibility, were targeted to investigate their modes of inheritance and to assess the scope for improving them relative to a base population. From a full-sib (FS) base population of pearl millet variety 'ICMV 221' three high and low nitrogen and three high and low *in vitro* digestibility FS were selected. Crosses were made for high × high (H × H), low × low (L × L) and high × low (H × L) FS trait contrasts and evaluated at Patancheru in the rainy seasons of 2007 and 2008. The high and low nitrogen FS parents contrasted 0.85 and 0.72% for nitrogen (N). In the crosses stover N contents were: HN × HN = 0.85%, LN × LN = 0.73% and HN × LN = 0.80% (P < 0.05). The high and low digestibility FS parents contrasted 43.3 and 40.3% for *in vitro* digestibility. In the crosses stover *in vitro* digestibilities (D) were: HD × HD = 43.7%, LD × LD = 40.3% and HD × LD = 42.2% (*P* < 0.05). The intermediate results of H × L crosses strongly indicates the additive nature of the stover quality traits nitrogen and *in vitro* digestibility and suggest the application of cyclic breeding methods for increasing stover nitrogen content and *in vitro* digestibility in pearl millet.

INTRODUCTION

In India, 66% of livestock are located in areas of rainfed agriculture and the bulk of their feed comes from crop residues (NIANP 2003; Ramachandra *et al*. 2007). Lack of feed quantity and quality are serious constraints to higher benefits from these livestock (Parthasarathy Rao and Birthal 2008). Pearl millet (*Pennisetum glaucum*) is a major crop in rainfed agriculture, contributing grain to food security and stover to livestock feed resources (Rachie and Majmudar 1980). The latter is especially important where (1) the dry season is too long (≥ 6 months) for native pasture resources to maintain animals until the next rainy season, and/or (2) an increased population density has drastically reduced the area of fallow/common property land that traditionally provided dry season grazing.

While the fodder quality of cereal stover is often low it was argued that even moderate increases in stover quality can have substantial effects on livestock productivity (Kristjianson and Zerbini 1999). Expression of stover quality traits such as stover nitrogen content and stover *in vitro* digestibility in pearl millet is complex (Hash *et al.* 2003) and efficient improvement in fodder value requires the understanding of the modes of its component trait inheritance. The objective of this study was to understand better the nature of gene action involved in inheritance of stover nitrogen and *in vitro* digestibility and to elucidate the scope for improving these two traits, using as a base population an elite open-pollinated variety of this highly heterogeneous and heterozygous out-crossing cereal.

MATERIALS AND METHODS

Breeding approach

The widely adapted pearl millet variety 'ICMV 221' (Witcombe *et al.* 1997) was chosen as a base population (C0 Bulk) for full-sib (FS) recurrent selection at ICRISAT in Patancheru. Based on their performance in the first rainy season trial in 2005 ten FS each for high and low stover nitrogen (N) and for high and low *in vitro* digestibility (IVOMD) were chosen from 256 FS progenies from the 'ICMV 221' base population. These selected sib-mated FS progenies were evaluated again in 2006 in an alpha-lattice design trial to select the three highest and three lowest FS for stover nitrogen content and for *in vitro* digestibility. The following crosses were made: high × high (H × H), low × low (L × L) and high × low (H × L) between the selected FS sets for stover nitrogen content and for *in vitro* digestibility, separately in summer 2006-07 by manual pollination of main stem panicles. Panicles were harvested and threshed individually. Cross types were: 1) High × High (H × H): H1 × H1, H1 × H2, H1 × H3, H2 × H2, H2 × H3 and H3 × H3, 2) Low × Low (L × L): L1 × L1, L1 × L2, L1 × L3, L2 × L2, L2 × L3 and L3 × L3, and 3) High × Low (H × L): H1 × L1, H1 × L2, H1 × L3, H2 × L1, H2 × L2, H2 × L3, H3 × L1, H3 × L2 and H3 × L3. These produced 21 crosses (combining reciprocal crosses to make a single entry) for each of the stover nitrogen and digestibility FS selections. FS crosses 6 (H × H), 6 (L × L) and 9 (H × L) and their parental FS progenies (H1, H2, H3, L1, L2 and L3) along with the base population (C0 Bulk) were evaluated under strictly rain-fed conditions in 2007 and 2008 at Patancheru, India.

Field trials

The entries in each FS set (stover N and stover *in vitro* digestibility) were grown separately in 4 blocks per replication and 7 entries in each block of alpha-lattice design trials with 6 replications. The crop was over sown and thinned to a uniform stand (10–12 cm between plants in a row). The crop received 17 kg ha^{-1} of both N and P in the form of 28-28-0 as basal fertilizer, and a side dressing of 100 kg ha^{-1} of urea 22 days after sowing. One hand weeding at the time of thinning was followed by 2 inter-culture operations to control weeds. Data on flowering time (50% stigma emergence on 50% of main stem panicles), panicle yield and dry stover yield were determined from 15 representative plants collected from each

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Table 1 Stover nitrogen (N) content, dry panicle (PY) and stover (SY) yield, stover *in vitro* digestibility (IVOMD), days to 50% flowering (DF$_{50}$) and digestible stover yield (DSY) in the original base populations (ICMV 221 C0 Bulk), high (H) and low (L) N parental FS progenies, and their respective crosses.

Entry	N (%)	PY (t ha^{-1})	SY (t ha^{-1})	IVOMD (%)	DF$_{50}$ (d)	DSY (t ha^{-1})
C0 Bulk	0.74	3.934	3.226	42.9	43.0	1.384
HN	0.85	3.121	2.576	41.7	42.5	1.074
LN	0.72	3.724	3.695	42.3	47.1	1.564
HN × HN	0.84	3.302	2.822	42.1	42.6	1.189
LN × LN	0.73	3.889	3.570	42.4	45.3	1.513
HN × LN	0.80	3.760	3.129	41.5	43.8	1.299
LSD (5%)	0.05	0.277	0.356	0.42	1.5	0.157
Correlations (r) between stover nitrogen and PY, SY, stover IVOMD, DF$_{50}$, DSY						
Stover N		-0.88	-0.95	-0.67	-0.76	-0.96
		[P = 0.02]	[P = 0.004]	[P = 0.154]	[P = 0.080]	[P = 0.002]

Table 2 Stover *in vitro* digestibility (IVOMD), dry panicle (PY) and stover (SY) yield, stover nitrogen (N), days to 50% flowering (DF$_{50}$) and digestible stover yield (DSY) in the original base populations (ICMV 221 C0 Bulks), high (H) and low (L) digestibility (D) parental FS progenies and their respective crosses.

Entry	IVOMD (%)	PY (t ha^{-1})	SY (t ha^{-1})	N (%)	DF$_{50}$ (d)	DSY (t ha^{-1})
C0 bulk	42.7	3.905	3.497	0.71	44.0	1.494
HD	43.3	3.315	3.862	0.73	44.9	1.670
LD	40.3	3.164	2.895	0.85	44.0	1.168
HD x HD	43.7	3.618	4.122	0.72	44.7	1.803
LD x LD	40.3	3.498	2.983	0.81	43.9	1.203
HD x LD	42.2	3.765	3.775	0.76	43.8	1.594
LSD (5%)	1.2	0.231	0.401	0.06	0.4	0.213
Correlations (r) between stover IVOMD and PY, SY, Stover N, DF$_{50}$, DSY						
IVOMD		-0.42	0.95	-0.93	-0.68	0.97
		[P =0.40]	[P = 0.003]	[P = 0.006]	[P = 0.13]	[P = 0.001]

individual plot. Plot values of panicle and stover yield were transformed to units of kg ha^{-1}.

Analysis of stover nitrogen and *in vitro* digestibility

Stover from 15 representative plants from each plot was dried and ground to pass a 1 mm sieve. Stover nitrogen and *in vitro* digestibility was estimated based on Near Infrared Spectroscopy (NIRS). The NIRS instrument used was a FOSS Forage Analyzer 5000 with software package WinISI. For conventional analysis to develop and validate the NIRS equations nitrogen (N) was estimated by auto analyzer. For analysis of *in vitro* digestibility (IVOMD) stover samples were incubated in rumen inoculum and *in vitro* organic matter digestibility was calculated based on gas volume produced after 24 h of incubation following Menke and Steingass (1988). The procedures and NIRS equations for predicting nitrogen content and *in vitro* digestibility in pearl millet stover have been described by Bidinger and Blümmel (2007). Digestible stover yield (DSY) was calculated by multiplying IVOMD (%) with stover yield (kg ha^{-1}).

Statistical analysis

Statistical analysis of the data used a general linear model implemented by SAS PROC GLM (Version 9.2; SAS Institute, Inc., Cary, NC). To facilitate the comparison between the base population, FS parents and FS crosses, least significant differences (LSD) at 5% level of significance were determined. Simple correlations of stover nitrogen with other measured traits for the stover nitrogen set of FS and similarly correlations of stover digestibility (IVOMD) with other measured traits for the digestibility set of FS were calculated based on mean data of 2007 and 2008 using SAS PROC CORR (Version 9.2; SAS Institute, Inc., Cary, NC).

RESULTS AND DISCUSSION

Stover nitrogen

The high and low nitrogen (N) parental FS progenies contrasted 0.85 and 0.72% for N (**Table 1**), showed a 15.5% increase and 2.6% decrease relative to the C0 Bulk (0.74% N), respectively. The HN × HN and LN × LN crosses had

0.84% and 0.73% stover N content. The HN × LN cross had an intermediate stover N content (0.80%), which clearly suggests the predominance of additive gene action. The additive effects of genes are fixable (Sprague and Tatum 1942) and are generally attributed to good general combining ability (*gca*). Selection of parents based on their *gca* effects is of great importance in breeding programs. However, breeding for increased stover N content is associated with penalties for panicle and stover yield, and hence low DSY (**Table 1**). Panicle and stover yields in the HN FS were significantly lower than in the C0 Bulk and in the LN FS. In conditions where stover yield is of lesser important than stover N content, crosses between the HN and LN parental FS (HN × LN) might be a good option since these crosses did not appear to reduce panicle yields relative to those of their LN parental FS while significantly increasing stover N (0.80% versus 0.72%). Low N content is often considered as primary constraint particularly in cereal crop residues, because rumen microbes require a minimum of about 1 to 1.2% of N in the diet otherwise feed intake and utilization might be severely depressed (Van Soest 1994). While increasing pearl millet stover N content from 0.72 to 0.80% would likely have a positive effect on feed intake (Ravi *et al*. 2010), additional protein supplementation would still be required to meet minimum microbial N requirements. Considering the significant general inverse relationship found between pearl millet stover N and panicle (r = -0.88) and stover (r = -0.95) yields and the fact that even stover improved for N would still not meet microbial nitrogen requirements, pursuing stover improvement for N seems questionable.

Panicle and stover yields might also have been affected by changes in flowering time. The LN parental FS progenies had flowering time delayed by 4 days (**Table 1**) relative to the base population (C0 Bulk), resulting in a longer vegetative growth phase and increased stover yield. In contrast the HN parental FS progenies flowered half a day earlier than the base population and had lower stover and panicle yields. A decrease in panicle-sink size is expected to accompany increased N concentration in the stover (compared to the base population) from the concept of interdependence of shoot and root function (Raper *et al*. 1978). Nitrogen uptake from the soil depends largely upon ade-

quate supply of carbohydrates translocated to roots from vegetative tissues (Jackson *et al.* 1980, 1986; Moll *et al.* 1994). So, it appears that early flowering of the HN parental FS progenies impaired their vegetative growth which could not produce sufficient photo-synthate to fully satisfy the parallel demands for carbohydrates from roots and panicles. In nitrogen remobilization study in durum wheat with high and low grain-stover protein selections showed that high protein selections had not only high N uptake in post-anthesis period but also had significantly lower N remobilization from vegetative organs to the grain compared to low protein selections (Suprayogi *et al.* 2011). Therefore, another possibility is lower sink strength of the HN FS, since in many crops grain yields obtained are conditioned by the amount of N that can be stored in the plants; materials with lower sink strength would likely tend to remobilize less of the accumulated N to the reproductive tissue, leading to higher N content of the stover.

Stover *in vitro* digestibility

Stover of the high and low digestibility (D) parental FS progenies contrasted 43.3% and 40.3% for IVOMD (**Table 2**), showed 1.4% increase and 5.6% decrease relative to the C0 Bulk. The HD × LD (42.2%) cross was positioned between the HD × HD and LD × LD crosses, again suggests the presence of additive gene action for stover IVOMD. Application of cyclic breeding methods, which involve selection and recombination among progenies to enhance the frequency of desirable alleles, maybe the best way to improve this trait. Cross HD × HD had one-percentage unit higher *in vitro* digestibility than the base population (43.7% versus 42.7%). These differences appear minor but as pointed out by Kristjanson and Zerbini (1999) a one-percentage unit increase in digestibility in sorghum and pearl millet stover could result in increased milk, meat or draught power outputs ranging from 6 to 8%. Blümmel and Rao (2006) observed that a one-percentage unit difference in *in vitro* digestibility of sorghum stover traded by fodder shops resulted in a price difference of about 5%.

CONCLUSION

Stover N content and *in vitro* digestibility were both largely governed by additive gene action in the pearl millet base population investigated and can therefore be improved through recurrent selection. Results indicated that stover digestibility, stover yield and time to flowering are linked traits indicating perhaps variability in particular partitioning cascades.

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