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Genetic Enhancement for Superior Food-Feed Traits in a Pearl Millet (*Pennisetum glaucum* (L.) R. Br.) Variety by Recurrent Selection

F.R. Bidinger (the Late)[1], M. Blümmel[2], C.T. Hash[1] and S. Choudhary[1,2]

[1]International Crops Research Institute for the Semi-Arid Tropics
Patancheru-502 324, India

[2]International Livestock Research Institute
Patancheru-502 324, India

ABSTRACT

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Grain and stover productivity and variation in stover quality traits were investigated amongst 256 full-sib progenies of pearl millet variety ICMV 221. Substantial ranges were observed for grain (1.5-fold difference) and stover (1.8-fold difference) yields. Stover protein content varied two-fold, ranging from 4.3to 8.6% and stover *in vitro* digestibilities ranged from 40.7 to 46.1%. Yield of digestible stover ranged from 1132 to 2388 kg ha^{-1}. From these full-sibs experimental varieties were generated targeting through two selection cycles: a) grain yield, b) dual-purpose usage, c) high stover protein; and d) high stover digestibility. Stover from each of the two selection cycles in 2005 and 2006 were tested with sheep for digestibility, intake, digestible organic matter intake and nitrogen balances. Generally traits in the experimental varieties were consistent with their intentional design. Through full-sib recurrent selection considerable gains were achieved in food-feed traits. For example more than a 17% increase was found in digestible organic matter intake in the dual-purpose experimental variety (15.1g Kg^{-1} LW d^{-1}) compared to the original variety (12.9 g Kg^{-1} LW d^{-1}). Similarly, nitrogen balance improved from -0.016 g Kg^{-1} LW d^{-1} in the sheep fed on stover from the original variety to +0.051 g Kg^{-1} LW d^{-1} in sheep fed stover from the experimental dual-purpose variety.

Key words: Recurrent selection, Pearl millet stover, Digestibility, Protein, Digestible organic matter intake.

INTRODUCTION

Several paper in this special issue discussed options for making use of variation in food-feed traits in existing cultivars (Blümmel *et al.,* 2010; Nigam and Blümmel, 2010). In addition, substantial impact could be achieved for future mixed crop-livestock

*Reprint request: Dr. M. Blümmel, E-mail: m.blummel@cgiar.org

systems when food-feed traits were further improved through targeted genetic enhancement (Kristjanson and Zerbini, 1999; Yadav and Bidinger, 2008). In other words, improving existing superior food-feed type cultivars even further through conventional (Bidinger *et al.* (2006) or maker assisted (Nepolean *et al.,* 2009) breeding. Hash *et al.,* 2006 found stable genetic variability in pearl millet for major quality traits (apart from nitrogen content) and Hall *et al.* (2004) reported high broad-sense heritability (H^2=0.7) for stover quality traits in pearl millet suggesting scope for further improvement of stover quality traits along with productivity. Therefore, the objectives of the present work were three-fold: first, to determine the ranges in grain yields and stover yields and stover quality traits in full-sib progenies of pearl millet cultivar ICMV 221. The widely adapted pearl millet variety ICMV 221 (Witcombe, *et al.,* 1997) was chosen as a base population for two cycles of full-sib progeny recurrent selection to assess the opportunities for improving stover nutritional quality of farmer-accepted open-pollinated cultivars. Second, to assess changes in grain and stover characteristics in trait-specific experimental varieties generated by random-mating selected subsets of these full-sib progenies; and third, to compare laboratory estimates of stover quality traits used for selection among progenies in the full-sib population with direct measurements of stover quality traits obtained through animal feeding trials, using stover samples from replicated field trials of the experimental varieties.

MATERIALS AND METHODS

Selection of progenies for experimental varieties

Two hundred and fifty-six full-sib progenies produced by plant × plant crosses of typical plants in pearl millet variety ICMV 221 were evaluated for agronomic characters, grain yield and stover yield in an alpha-lattice design trial with 2 replications and 36 blocks within each replications conducted at ICRISAT-Patancheru during the rainy season of 2004 (Temp 15.3-29.5^{0}C and RH 38-98% on average basis of day and Night) under completely rain-fed condition. 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. Data on flowering grain yield and stover yield were collected on an individual plot basis. Plot values of grain and stover yield were transformed to units of kg ha^{-1} and analyzed with the Residual Maximium Likelihood (REML) algorithm of the GenStat statistical package (Rothamsted, UK) to obtain the estimates of the agronomic performance of each full-sib progeny. Representative dry stover samples from each plot were ground to a fine powder and analyzed for quality traits. Based on the analyzed full-sib progeny data sets for agronomic and stover quality traits, full-sibs were selected as parents for four experimental varieties as follows:

- High grain yield variety: 16 full-sib progenies selected based on: 1) time to flowering within mean ±2 SD; 2) grain yield ≥ mean; 3) stover yield ≥ mean –1 SD; 4) nitrogen content and *in vitro* digestibility ≥ mean +0.5 SD.

- Dual-purpose variety: 16 full-sib progenies selected based on: 1) time to flowering within mean ±2 SD; 2) stover yield ≥ mean; 3) grain yield ≥ mean –1SD; 4) nitrogen content and *in vitro* digestibility ≥ mean +0.6 SD.
- High nitrogen variety: 15 full-sib progenies selected based on: 1) time to flowering within mean ±2 SD; 2) ranks for nitrogen content.
- High digestibility variety: 15 full-sib progenies selected based on: 1) time to flowering within mean ±2 SD; 2) ranks for *in vitro* digestibility.

Remnant seed of the selected full-sib progenies were sown in an off-season nursery and the progenies in each group random-mated manually to produce Syn0 generation seed of the four experimental varieties. An unselected sample of the base population, ICMV 221, was also random-mated manually to provide an appropriate control entry.

Stover quality analysis

Stover fodder quality was analyzed by a combination of conventional laboratory analysis and Near Infrared Spectroscopy. Near Infrared Spectroscopy was calibrated for stover nitrogen content as measured by auto analyzer and for stover *in vitro* digestibility as measured by incubation in rumen inoculum (Menke and Steingass, 1988). Neutral detergent fiber (NDF) an estimate of the cell wall fraction, acid detergent fiber (ADF) an estimate of cellulose content and acid detergent lignin (ADL) were determined by routine chemical analytical procedures (Van Soest and Robertson, 1985).

Agronomic assessment of experimental varieties and feeding trials

The four experimental varieties and unselected control variety of ICMV 221 were grown in a randomized complete block design with two field replications of large plots (10 rows of 80 m length), at ICRISAT-Patancheru during the rainy season of 2005 (Temp 14.7-28.5 ^{0}C and RH 41-100%) and 2006(Temp 15.8-29.6 ^{0}C and RH 51-99%). The trials were conducted on an Alfisol, over-sown and thinned to a uniform stand, and managed similarly to the full-sib progeny trial of previous rainy season.Panicle and stover from each plot were harvested separately, panicle from each plot threshed manully. Stover was chopped and dried in drying bins.

Growing male Deccani sheep of mean live weight of about 20 kg were used for the *in vivo* feeding experiments. The sheep were housed in metabolic cages enabling the measurements of feed intake, feed digestibility and nitrogen balances. Each stover was fed to 12 sheep randomly allocated according to body weight since sheep groups were balanced according to live weight. The sheep were accustomed to a stover for a minimum of two weeks, which was then followed by a 10-days faecal and urine collection period for estimation of digestibility and nitrogen balance respectively. All stovers were offered chopped and as sole feed of about 15 per cent of appetite. In other words sheep were allowed to refuse about (15%) of the stover offered, which is the most common norm when investigating voluntary (*ad libitum*) feed intake.

RESULTS AND DISCUSSION

Variations in grain and stover traits in 256 full-sib progenies of ICMV 221

Variation in grain and stover productivity and variation in stover quality traits among the 256 full-sib progenies of ICMV 221 were reported in Table 1. Substantial ranges were observed for grain (1.5-fold difference) and stover (1.8-fold difference) yields. Stover protein content varied two-fold, ranging from 4.3%, which presents a highly deficient protein status, to 8.6%, which is more than the minimum (about 7%) feed protein requirement of rumen microbes (Van Soest, 1994). Similarly, yield of digestible stover, which is the product of stover yield and stover *in vitro* digestibility, ranged from 1132 to 2388 kg ha^{-1} — more than a two-fold difference (Table 1). Stover *in vitro* digestibilities varied by 5.4 percentage units, and this magnitude in pearl millet stover quality difference within a full-sib population appears considerable. Kristjanson and Zerbini (1999) calculated that one-percentage unit increase in digestibility in sorghum and pearl millet stover would result in increases in milk, meat and draught power outputs ranging from 6 to 8 per cent. These estimates appear very high, but they are broadly supported by observed differences in prices of traded sorghum stover where a difference in digestibility of 5% was associated with price premiums of 30% and higher (Blümmel and Rao, 2006). In a total of 16 landraces, dual-purpose and hybrids pearl millet cultivars subjected to different management systems over 2 years. Blümmel *et al.* (2007) observed a 3.8% difference in *in vitro* digestibility. These diverse observations about variations in stover *in vitro* digestibility suggest the 5.4% difference found in the full-sib population to be sufficient to select further for this trait. Clearly considerable scope exists within an open-pollinated variety such as ICMV 221 for selection of experimental varieties having contrasting grain and stover characteristics.

Variation in grain and stover traits among four experimental varieties selected from ICMV 221

Except for stover yield in 2005, biomass and grain yields were not statistically different ($P > 0.05$) across ICMV 221 Original and experimental varieties (Table 2). The fact that few significant differences were found for grain yields between the five varieties

Table 1. Summary of variation in food-feed crop traits within 256 full-sib progenies of pearl millet cultivar ICMV 221, ICRISAT, Patancheru, rainy season 2004

Variable	Mean	Range
Grain yield (kg ha^{-1})	3561	2719 to 4154**
Stover yield (kg ha^{-1})	3617	2783 to 5005**
Stover protein (%)	6.2	4.3 to 8.6**
Stover *in vitro* digestibility (%)	43.6	40.7 to 46.1**
Digestible stover yield (kg ha^{-1})	1577	1132 to 2388**

**Maximum and minimum values are significantly different ($P < 0.01$.)

Table 2. Biomass, grain and stover yields of control and of four experimental varieties (kg ha^{-1}) selected from ICMV 221, Patancheru, India, rainy seasons 2005 and 2006

Cultivar type	Biomass yield		Grain yield		Stover yield	
	2005	2006	2005	2006	2005	2006
ICMV 221 Original	7097		3105		3138^{b}	
High Grain Yield	6988	5403	3109	2001	3051^{b}	2534
Dual-purpose	7617	5288	3248	2256	3499^{a}	2277
High Nitrogen	6675	5130	2992	2176	2892^{b}	2170
High Digestibility	6582	5788	2862	2263	2945^{b}	2669
P	ns	ns	ns	ns	0.007	ns

Values for a trait that are followed by different superscripts are different; ($P<0.05$).
ns=Non significant.

(Table 2) suggests that genetic enhancement of stover quality would not adversely affect grain yield. Hash *et al.* (2006) also showed lack of negative relationship between stover quality and grain and stover yield in 30 diverse pearl millet cultivars. The two stover quality traits were directly targeted by the selection process, high nitrogen content and high digestibility, and the results reported in (Table 3) are consistent with the intentional design of the experimental varieties as far as stover nitrogen is concerned. Stover of the high nitrogen variety did have, in fact, the highest nitrogen content in both years but differences among the experimental varieties were insignificant which indicated stover nitrogen largely determined by environment (Bidinger and Blümmel, 2006). Chakrobarty *et al.* (2009) reported crude protein content in rice grain with high heritability but slow genetic advance because of high environment influence. In 2005 the high digestibility variety had the highest *in vitro* digestibility and in 2006 the second highest digestibility.

One of the traits the experimental varieties were designed for, namely high digestibility should be reflected in the results of the feeding trial. For 2005 this was indeed the case and the *in vivo* digestibility of this variety was 57.5%, significantly higher than of any of the other four varieties (Table 4). No significant differences in *in vivo* digestibility between the varieties were observed in 2006 but both the dual-purpose and the high digestibility variety tended ($P=0.06$) to showed higher *in vivo* digestibilites than any of the other varieties. These two experimental varieties were also superior in digestible organic matter intake (DOMI) and nitrogen balances with the dual-purpose experimental variety significantly out performing all others (Table 4). If the control variety (ICMV 221 Original) is taken as the point of departure for DOMI, two rounds of selection have resulted in an increase of 2.2 g kg^{-1} live weight per day (LW d^{-1}), which is more than 17%, which seems remarkable.

The high nitrogen experimental variety indeed had the highest nitrogen content (Table 3) but feed nitrogen does not automatically translate into nitrogen retention in the

Table 3. Percentage of stover nitrogen content, *in vitro* digestibility, neutral detergent fiber (NDF) and acid detergent fiber (ADF) and acid detergent lignin (ADL) in stover of a control and four experimental varieties of ICMV 221 produced at ICRISAT-Patancheru, rainy season 2005 and 2006.

Cultivar type	Nitrogen (%)		*In vitro* digestibility (%)		NDF (%)		ADF (%)		ADL (%)	
	2005	2006	2005	2006	2005	2006	2005	2006	2005	2006
ICMV 221 Original	0.90[b]		47.9		69.6[b]		40.8[a]		5.9[a]	
High Grain Yield	0.93[b]	0.91	46.9	49.4	70.5[a]	71.7	40.2[c]	37.2	5.6[bc]	4.4
Dual-purpose	0.91[b]	0.88	47.9	50.4	69.6[b]	70.6	40.2[c]	36.3	5.5[cd]	4.0
High Nitrogen	0.99[a]	0.95	46.7	50.8	71.1[a]	70.2	41.7[a]	37.7	5.8[ab]	4.2
High Digestibility	0.90[b]	0.83	48.1	50.5	67.7[c]	70.8	39.7[d]	38.5	5.2[d]	4.4
P	0.03	ns	ns	ns	< 0.0001	ns	< 0.0001	ns	0.0004	ns

Table 4. Organic matter intake (OMI), organic matter digestibility (OMD), digestible organic matter intake (DOMI) and nitrogen balance in sheep fed stover of a control and four experimental varieties of ICMV 221, produced at ICRISAT-Patancheru, rainy season 2005 and 2006.

Cultivar type	OMI (g Kg^{-1} LW d^{-1})		OMD (%)		DOMI (g Kg^{-1} LW d^{-1})		N-Bal. (g Kg^{-1} LW d^{-1})	
	2005	2006	2005	2006	2005	2006	2005	2006
ICMV 221 Original	23.8		54.4[b]		12.9		-0.016	
High Grain Yield	24.2	23.0[b]	54.1[b]	55.4	13.1	12.7[b]	0.014	0.017[b]
Dual-purpose	25.5	25.9[a]	56.6[a]	58.7	14.4	15.1[a]	-0.016	0.051[a]
High Nitrogen	24.0	23.7[b]	54.0[b]	55.4	13.0	13.1[b]	0.006	0.028[b]
High Digestibility	24.9	24.6[ab]	57.5[a]	55.7	14.3	13.6[b]	0.023	0.022[b]
P	ns	0.05	<0.001	0.06	ns	<0.0001	ns	0.0005

Values for a trait that are followed by different letters are different ($P<0.05$).

animal, as energy is required for nitrogen accretion as lean tissue, and this energy comes from the digestible matter. However, the over all level of nitrogen inputs and outputs in this feeding experiment were relatively small, and the potential for analytical errors were considerable because feed nitrogen, feed refusals nitrogen, urinary nitrogen and faecal nitrogen are all input variables into the balance. The findings of the nitrogen balance should therefore be taken with some caution. Still the dual-purpose experimental variety had significantly higher nitrogen balance than the other varieties probably as a result of having superior DOMI (Table 4).

CONCLUSION

The results based on initial assessment of food-feed quality trait in pearl millet proved full-sib recurrent selection to be an effective breeding method in improvement of complex quantitative traits (like digestibility), probably because it gave complete control on parents and allowed the accumulation of favourable alleles while maintaining genetic variability through intermating within a population. Overall, the results from this initial assessment of response to recurrent selection for improved ruminant livestock feeding value of stover of pearl millet variety ICMV 221 were encouraging since stover fodder quality could be increased without detriment to grain and stover yield.

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