

## QTLs Controlling Yield and Stover Quality Traits in Pearl Millet

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### Introduction

Stover of pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a major source of dry-season maintenance rations for livestock in traditional smallholder crop-livestock production systems on the semi-arid margins of the Thar desert in South Asia and in the Sahelian region of West and Central Africa (Renard 1997).

Crop residues, especially those produced in marginal environments, have poor nutritional value. Although nutritional supplements can enhance the feed quality of crop residues, they may not provide a long-term solution because of difficulties in adapting them to local conditions, limited availability, and expense. Genetic enhancement of crop-residue nutritional quality is an alternative approach that can naturally, cost-efficiently, and permanently improve productivity of these crop-livestock systems.

Expression of stover quality traits in pearl millet is complex (Hash et al. 2003), and selection based on conventional breeding methods is potentially difficult considering that conventional wet-lab analysis of nutritional value is tedious and time-consuming. Identification of genomic regions associated with stover quality traits would permit application of marker-assisted backcrossing (MABC) to improve the feed value of crop residues of elite hybrids and their parental lines.

Preliminary assessment of genetic variability of stover quality traits and stover yield in pearl millet has demonstrated the presence of considerable variation for cell wall digestibility and stover yield (Blümmel et al. 2003). The pearl millet mapping population based on the cross ICMB 841 × 863B used in this study is one of several developed for drought tolerance. It exhibits only limited variation for flowering time and plant height while varying substantially for many other plant traits and maintaining a reasonable degree of agronomic eliteness. Parents of this population exhibit inherent variation for many stover quality traits, so this population provides ample scope for quantitative trait locus (QTL) mapping and marker-assisted selection (MAS) (Hash et al. 2003). The present study had the following objectives: mapping QTLs for stover nutritional quality, assessing relationships among

stover yield components and quality traits, and selecting target QTLs for MABC. This is the first detailed report of QTLs mapped for pearl millet stover ruminant nutritional quality traits.

### Materials and Methods

A set of 149 F<sub>2</sub>-derived F<sub>4</sub> self-bulk progenies of a pearl millet drought tolerance mapping population based on cross ICMB 841 × 863B was used to map QTLs controlling stover quality traits. Parent 863B was bred at ICRISAT-Patancheru, India from an *Iniadi* landrace from Togo, and was initially chosen on the basis of its combination of agronomic eliteness and superior combining ability for terminal drought tolerance. Parent ICMB 841 is also agronomically elite but has poor combining ability for terminal drought tolerance. Compared to 863B it exhibits inferior combining ability for several stover quality traits (Hash et al. 2003). The F<sub>2</sub>-derived F<sub>4</sub> progenies of this mapping population were crossed to tester H 77/833-2 producing testcross hybrids, which were evaluated at ICRISAT-Patancheru in a replicated alpha-design trial during the rainy season of 2005. The experiment was conducted in 2-row plots of 4-m length, with the rows spaced 75 cm apart. The crop was grown following normal agronomic practices. It was completely rainfed and suffered no significant disease or insect damage.

Whole plant stover samples of each testcross hybrid were collected from 15 plants per plot at physiological maturity. Samples were oven-dried, weighed and finely ground prior to quality assessment. Ground stover samples were subjected to Near Infrared Reflectance Spectroscopic (NIRS) analysis, and predictive equations were developed for all laboratory estimates of stover quality traits studied. These included gas volume (mL) produced after 24 h of *in vitro* digestion of 200 mg dry matter (GAS24), and *in vitro* organic matter digestibility (IVOMD), nitrogen content (NDM), metabolizable energy content (ME), and sugar content (SUGSDM) on dry matter basis. Predictions on the basis of NIRS analysis for these traits from each plot were then used for further analysis. The mean values

for these quality traits, as well as the dry stover yield (DSY) and grain yield (GY) of the testcross hybrids, were determined by the best linear unbiased prediction method using the ReML procedure within the GenStat statistical software package (Rothamsted, UK). The predicted testcross means of each of these traits were then used in QTL mapping.

Several new PCR-compatible markers were added to the pre-existing linkage map of this population (Yadav et al. 2004), which was based on polymorphic RFLP and SSR markers. New marker loci were assigned to linkage groups based on marker recombination and ordered within linkage groups to minimize the frequency of candidate errors. After removing collinear markers, seven linkage groups were constructed with data from 95 polymorphic RFLP, SSR, TRAP and EST-SSR loci using MAPMAKER/Exp (Lander et al. 1987). Genotypic and phenotypic data sets were then fed into PLABQTL 1.1 (Utz and Melchinger 1996) to perform composite interval mapping analysis.

## Results and Discussion

The total map length obtained was 1058 cM (Haldane units) with an average interval length of 12 cM. Testcross hybrids expressed significant genetic variability for all observed stover quality traits (Table 1). Operational heritabilities based on entry means were above 50% for GAS24, IVOMD, ME, SUGSDM and DSY.

The QTL analysis performed for individual stover quality traits identified a region on linkage group 2 (LG 2) controlling GAS24, IVOMD, ME and SUGSDM (Table 2). This genomic region may pleiotropically control these traits, with the favorable allele in all cases being inherited from parent 863B. This was the most significant finding

in this mapping exercise, and has considerable implications for applied breeding. In previous studies using testcrosses of this same mapping population (Yadav et al. 2004), this genomic region of 863B was identified as a candidate for MAS to improve terminal drought tolerance of ICMB 841B and its hybrids. Thus, transferring this stover quality QTL from 863B to 841B and other elite hybrid parental lines should also improve the terminal drought tolerance of their hybrids. Considering the geographical region where dual-purpose pearl millet genotypes are grown, this would be highly beneficial.

A second genomic region on LG 6 controlled a significant proportion of the phenotypic variances for all quality traits considered in this study, with all except NDM favored by 863B alleles. For GAS24 and ME, a third QTL was mapped on LG 4 near to a major downy mildew resistance locus where 863B provides the superior allele for disease resistance (Hash and Thakur, unpublished). For IVOMD a QTL was mapped at the top of the LG 1, which also appeared to control GAS24 and ME (Table 2).

Three QTLs were identified for dry stover yield. These genomic regions were distributed across LG 3, LG 5 and LG 6 and together controlled a significant proportion of observed phenotypic variance for dry stover yield among the testcrosses. The favorable alleles for QTLs mapped on LG 3 and LG 6 were provided by 863B, whereas alleles of ICMB 841 were favorable for the QTL on LG 5. This QTL on LG 5 for dry stover yield co-mapped with QTLs for NDM and SUGSDM. The testcrosses of this mapping population were also used to identify grain yield QTLs, with LG 5 being found to contribute substantially to grain yield variation as it harbors three grain yield QTLs. The grain yield QTL at the bottom of the LG 5 mapped near to those for dry stover yield, NDM

**Table 1. Means and variances of 149 testcrosses of the (ICMB 841 × 863B)-derived pearl millet mapping population for stover quality and yield traits<sup>1</sup>, ICRISAT-Patancheru, India, rainy season 2005.**

	GAS24 (mL)	IVOMD (% of DM)	ME (MJ kg <sup>-1</sup> DM)	NDM (% of DM)	SUGSDM (% of DM)	DSY (kg ha <sup>-1</sup> )	GY (kg ha <sup>-1</sup> )
Minimum	20.3	36.6	5.23	0.33	0.83	998.0	988.0
Maximum	33.4	48.0	6.93	1.20	8.17	3212.0	2988.0
Mean	26.9	42.5	6.11	0.67	3.71	2029.0	1921.0
SE (±)	1.19	0.96	0.15	0.06	0.68	158.24	169.24
CV (%)	7.7	3.9	4.3	16.2	31.6	13.5	15.3
F ratio	2.31	2.03	2.24	1.67	2.18	2.53	1.33
h <sup>2.2</sup> (%) <sup>2</sup>	30	26	29	18	28	34	10
h <sup>2.3</sup> (%) <sup>3</sup>	57	51	55	40	54	61	25

1. GAS24 = Gas volume produced after 24 h of *in vitro* digestion of 200 mg DM of ground sample; IVOMD: *in vitro* organic matter digestibility; NDM = nitrogen on dry matter basis; ME: metabolizable energy on dry matter basis; SUGSDM = sugar content on dry matter basis; DSY: dry stover yield; GY: grain yield; DM = dry matter.
2. h<sup>2.2</sup> = Operational heritability calculated on plot basis.
3. h<sup>2.3</sup> = Operational heritability calculated on entry mean basis.

**Table 2. QTLs detected for stover quality-related traits, dry stover yield and grain yield sharing common genetic regions.**

Trait <sup>1</sup>	LG	Marker interval	Position	LOD	R <sup>2</sup>	Additive effect
GAS24 (mL)	1	<i>Xbm1RA10a</i> – <i>Xpsm761</i>	3	3.5	10.8	0.422
	2	<i>Xpsmp2066</i> – <i>Xpsm380</i>	3	5.5	16.5	0.575
	4	<i>Xpsm1003d</i> – <i>Xpsm716</i>	18	3.1	10.1	0.379
	6	<i>Xpsm514</i> – <i>XtstRA6c</i>	0	4.5	13.8	0.520
IVOMD (% DM)	1	<i>Xpsm761</i> – <i>Xbm3RA1c</i>	1	3.1	9.7	0.294
	2	<i>Xpsmp2066</i> – <i>Xpsm380</i>	3	4.0	12.2	0.341
	6	<i>Xpsm514</i> – <i>XtstRA6c</i>	0	4.2	12.8	0.348
ME (MJ kg <sup>-1</sup> DM)	1	<i>Xbm1RA10a</i> – <i>Xpsm761</i>	3	3.3	10.2	0.053
	2	<i>Xpsmp2066</i> – <i>Xpsm380</i>	3	4.1	12.5	0.063
	6	<i>XtstRA6c</i> – <i>Xicmp3002</i>	1	3.1	9.6	0.059
NDM (% DM)	2	<i>Xpsmp2066</i> – <i>Xpsm380</i>	1	5.0	15.2	-0.018
	4	<i>Xpsm265</i> – <i>Xpsm344</i>	1	2.7	8.5	-0.012
	5	<i>Xctm25</i> – <i>Xpsmp2064</i>	10	5.3	15.9	0.027
	5	<i>Xpsms53</i> – <i>Xpsms70</i>	2	3.8	12.1	-0.022
	6	<i>Xpsmp2213</i> – <i>Xpsm713</i>	1	6.6	19.6	-0.019
SUGSDM (% DM)	2	<i>Xpsmp2066</i> – <i>Xpsm380</i>	1	4.2	13.0	0.268
	5	<i>Xpsm318</i> – <i>Xpsms18</i>	0	4.6	14.4	-0.236
	6	<i>XtstRA6c</i> – <i>Xicmp3002</i>	1	4.8	14.5	0.264
DSY (kg ha <sup>-1</sup> )	3	<i>Xctm10</i> – <i>Xpsm174</i>	17	5.0	14.9	74.1
	5	<i>Xpsmp2064</i> – <i>Xpsm318</i>	4	9.0	26.0	-104.9
	6	<i>XtstRA6c</i> – <i>Xicmp3002</i>	3	7.2	20.9	101.2
GY (kg ha <sup>-1</sup> )	5	<i>Xicmp3027</i> – <i>Xbm1RA1d</i>	0	3.2	10.4	39.1
	5	<i>Xbm1RA5a</i> – <i>Xbm3RA1d</i>	16	2.8	8.7	-39.2
	5	<i>Xpsms70</i> – <i>Xpsm732.1</i>	5	3.5	11.3	-25.7

1. GAS24 = gas volume produced after 24 h of *in vitro* digestion of 200 mg DM of ground sample; IVOMD = *in vitro* organic matter digestibility; NDM = nitrogen on dry matter basis; ME: metabolizable energy on dry matter basis; SUGSDM = sugar content on dry matter basis; DSY: dry stover yield; GY: grain yield; DM = dry matter; LG = linkage group; marker interval = loci flanking the QTL LOD peak; Position = distance (cM, Haldane units) of the QTL peak from the left-flanking marker; R<sup>2</sup> = percentage of phenotypic variance explained by the QTL. A positive sign of the additive effects indicates the 863B allele contributed favorably to the trait.

and SUGSDM, with ICMB 841 providing favorable alleles for all of these traits. Thus positive association makes this genomic region a potential target region for MAS.

The best phenotypic assessment of stover quality traits involves animal feeding experiments, which are expensive, laborious and time-consuming. NIRS predictions of these traits allows them to be addressed effectively in a breeding program (Bidinger et al. 2006), and when combined with QTL mapping should improve breeding efficacy. The improved understanding of associations between stover quality traits and molecular markers afforded by this study is expected to greatly facilitate improvement of these traits in elite hybrids and their parental lines.

Several QTLs identified in this study were consistent in their expression and controlled significant portions of the observed phenotypic variation for more than one trait. Most of the QTLs mapped for yield components and stover quality traits in this study were flanked by polymorphic PCR-compatible markers that could facilitate their rapid and efficient exploitation in MABC programs.

Development of improved dual-purpose pearl millet hybrids is the ultimate goal of this research. Looking at

the independent segregation of major QTLs detected in this study for grain yield, stover yield and stover quality, development of dual-purpose pearl millet hybrids having high grain and stover yields combined with good stover quality attributes should not be unduly difficult.

**Acknowledgment.** This document is an output from projects funded by the Australian Centre for International Agricultural Research (ACIAR) and by the Plant Sciences Research Programme of the UK Department for International Development (DFID). The latter (R8183) was administrated by the Centre for Arid Zone Studies (CAZS) for the benefit of developing countries. The views expressed are not necessarily those of ACIAR and DFID. The authors express their gratitude to M Mark, Jaihind, A Ganapathy and P Omprakash for their assistance with the field experiments, C Ramakrishna Reddy for his assistance with stover quality assessment, and M Narsi Reddy for his contribution to molecular marker data generation.

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