



Opportunities for marker-assisted selection (MAS) to improve the feed quality of crop residues in pearl millet and sorghum

C.T. Hash^{a,*}, A.G. Bhasker Raj^a, S. Lindup^b, A. Sharma^{a,c}, C.R. Beniwal^c,
R.T. Folkertsma^a, V. Mahalakshmi^a, E. Zerbini^{d,e}, M. Blümmel^d

^aInternational Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Andhra Pradesh, India

^bCambridge Laboratory, John Innes Centre (JIC), IPSR, Colney Lane, Norwich NR4 2UW, UK

^cDepartment of Plant Breeding, Chaudhary Charan Singh Haryana Agricultural University (CCSHAU),

Bajra Section, Hisar 125 004, Haryana, India

^dInternational Livestock Research Institute (ILRI), South Asia Project, Patancheru 502 324, Andhra Pradesh, India

^eCargill Animal Nutrition European Laboratory, Spessa, Italy

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Abstract

Cereal crop residues (straw, chaff, etc.) are important components of maintenance rations for ruminant livestock in many parts of the world. They are especially important in small-holder crop-livestock production systems in the sub-humid, semi-arid, and arid tropics and subtropics where most of the world's poorest livestock producers and consumers are found. Taking as examples tropically adapted cereals in the crop improvement mandate of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), namely pearl millet [*Pennisetum glaucum* (L.) R. Br.] and sorghum [*Sorghum bicolor* (L.) Moench], this paper explores opportunities for using marker-assisted crop breeding methods to improve the quality and quantity of cereal crop residues for use as ruminant livestock feedstuffs. In the case of pearl millet, ICRISAT has been heavily involved with several UK-based collaborating research institutes, in development and initial application of the molecular genetic tools for marker-assisted breeding. We have obtained some useful experience in quantitative trait loci (QTL) mapping and marker-assisted selection (MAS) for stover yield, foliar disease resistance, and in vitro estimates of the nutritive value of various stover fractions for ruminants. In sorghum, ICRISAT has focused on initiating a large-scale high-throughput marker-assisted backcrossing program for the stay-green component of terminal drought tolerance—a trait that is likely to be associated not only with more stable grain and stover yield, but which is also expected to contribute to maintenance of ruminant nutritional value of stover produced under drought stress conditions. Conventional and marker-assisted breeding for foliar disease resistance is recommended for dual-purpose cereal improvement, or indeed for improvement of the nutritional value of residues for any crop in which these are used as feedstuffs for ruminant livestock. Practical problems faced and proposed ways of dealing with these are discussed.

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1. Introduction

Cereal crop residues (straw, chaff, etc.) are an important component of maintenance rations for

* Corresponding author. Tel.: +91-40-2329-6161;

fax: +91-40-2324-1239.

E-mail address: c.t.hash@cgiar.org (C.T. Hash).

ruminant livestock in many parts of the world. They are especially important in small-holder crop-livestock production systems in the sub-humid, semi-arid, and arid tropics and subtropics where most of the world's poorest livestock producers and consumers are found. This paper explores opportunities for using marker-assisted crop breeding methods to improve the quality and quantity of cereal crop residues for use as ruminant livestock feedstuffs, taking as examples two tropically adapted cereals in the crop improvement mandate of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), namely pearl millet [*Pennisetum glaucum* (L.) R. Br.] and sorghum [*Sorghum bicolor* (L.) Moench]. ICRISAT has been pursuing slightly different strategies in marker-assisted selection (MAS) of these two crops, based largely on differences in molecular genetic technologies available from other research centers for sorghum and pearl millet, and the interests of specific research funding agencies to support our efforts in this area.

In case of pearl millet, ICRISAT has been heavily involved with several UK-based collaborating research institutes, in development and initial application of the molecular genetic tools for marker-assisted breeding. We have obtained some useful experience in quantitative trait loci (QTL) mapping and MAS for stover yield, foliar disease resistance, and in vitro estimates of the nutritive value of various stover fractions for ruminants. In case of sorghum, there have been many more laboratories globally with interests in developing the molecular genetic tools required. Hence we are concentrating on application of findings from these other labs and use of their tools to solve problems of greater interest to our national program partners and farmers. Thus in sorghum we have focused on initiating a large-scale high-throughput marker-assisted backcrossing program for the stay-green component of terminal drought tolerance—a trait that is likely to be associated not only with more stable grain and stover yield, but which is expected to also contribute to maintenance of ruminant nutritional value of stover produced under drought stress conditions. Although ICRISAT has not yet initiated work on marker-assisted improvement of foliar disease resistance in sorghum, our experience in pearl millet suggests that this should be a cost-effective way to improve ruminant

nutritional value of sorghum crop residues (or indeed those of any crop for which such residues find value as livestock feedstuffs) in the short-to-medium term.

2. Molecular genetic maps are the basis for marker-assisted plant breeding

Compared to better-studied cereals such as rice, wheat, maize, and barley, there has been relatively little research on the development and application of molecular genetic maps of pearl millet (Liu et al., 1994, 1996, 1997; Busso et al., 1995; Burton and Wilson, 1995; Jones et al., 1995, 2002; Morgan et al., 1998; Devos and Gale, 1997, 2000; Devos et al., 1998, 2000; Gale and Devos, 1998a,b; Poncet et al., 2000, 2002; Qi et al., 2001; Witcombe and Hash, 2000; Allouis et al., 2001; Bhattacharjee et al., 2002; Hash et al., 2002; Yadav et al., 2002, 2003). Most of this published work on the development and application of DNA-marker-based genetic linkage maps for pearl millet improvement has involved collaborative research programs involving two or more of the following public research institutions: John Innes Centre (JIC), Norwich, UK; ICRISAT, Patancheru, India; University of Paris-Sur, Paris, France; Centre for Arid Zone Studies (CAZS), University of Wales, Bangor, UK; Institute of Grassland and Environmental Research (IGER), Aberystwyth, UK; USDA, University of Georgia Coastal Plain Experiment Station, Tifton, USA; All-India Coordinated Pearl Millet Improvement Programme (AICPMIP) and/or state university pearl millet breeding programs in India. However, the relatively small number of publications and limited range of genetic tools has made it difficult to use this information to more efficiently exploit the tremendous range of genetic variation available for crop improvement in cultivated pearl millet and its wild and weedy relatives within *P. glaucum*.

There have been far more research players (in both public- and private-sector research organizations) in the USA, Europe, Australia, Latin America, Asia and Africa that have been involved in development and exploitation of molecular genetic maps for sorghum (e.g., Haussmann et al., 2002a). Unfortunately their efforts have not been particularly well-coordinated due to competition between the different groups of research organizations and scientists. Despite this,

compared to pearl millet there is a substantially larger number of refereed publications for sorghum in this area. These publications provide a larger and more diverse range of genetic tools for marker-assisted breeding in this less variable crop, as described below for the stay-green trait.

3. QTL mapping of stover yield and quality traits in pearl millet

This has been a substantial component of two competitive research grants involving ICRISAT and ILRI scientists based at ICRISAT, Patancheru, India. The first was an ACIAR-supported project (1998–1999) entitled “Development and use of molecular genetic markers for enhancing the feeding value of cereal crop residues for ruminants”. This included assessment of opportunities in existing pearl millet mapping populations and an ILRI/ICRISAT piggy-back stover quality component for a DFID/PSP-supported drought tolerance mapping study of testcross hybrids from a population based on ICMB 841 × 863B and involving ICRISAT, JIC and IGER. The second was a DFID hold-back project (1999–2002) entitled “Genetic enhancement of feed quality and quantity in sorghum and pearl millet” which included a component extending the previous ACIAR-supported assessment of available pearl millet mapping populations for variation in stover yield and quality, and stover quality mapping in an ICRISAT/IGER/JIC population based on ICMB 841 × 863B. Follow-up research, including completion of stover sample quality assessment and analysis of data sets generated in these projects, continues.

These two pearl millet stover quality mapping studies used replicated multi-locational field trials, with in vitro assessment of quality-related attributes of stover fractions (leaf blade, leaf sheath, and stem internode corresponding to the main stem’s penultimate leaf) of a 1) line × tester (L × T) trial set to assess, in the form of testcross hybrids on four genetically, cytoplasmically, and phenotypically diverse male-sterile lines, opportunities for using parents of the nine pearl millet mapping populations available at ICRISAT, Patancheru, India; and a 2) (ICMB 841 × 863B)-based mapping population progeny testcross trial, using genetically diverse testers H 77/833-2 and

PPMI 301 and a relatively small set of 79 mapping population progenies. Initial results from the pearl millet mapping population parents L × T study showed considerable genetic variation for grain and stover yield components, with expected genotype × environment interactions due to the differential photoperiod sensitivity of the parental lines used. Difficulties were encountered with conducting in vitro analyses for such large sample numbers, so initially only a portion of field replicates were analyzed. Results from this portion of the available field replicates indicated that operational heritabilities were low for in vitro measures of stover nutritional quality. The next steps are to complete chemical and in vitro analyses in combination with near-infrared spectroscopy (NIRS) for the remaining field replicates. Recent data thus obtained by Bidinger et al. (unpublished) suggest high heritabilities (>0.8) for in vitro digestibility in pearl millet stover. However, as pointed out by Blümmel et al. (2003a,b), these relationships may be misleading unless in vitro digestibility and other laboratory stover quality assessments are firmly linked to animal performance measurements such as milk production or live weight gains. These authors suggested that all laboratory quality assessments should be validated in the target crops by animal performance measurements before being applied in large-scale screening work.

None-the-less, two existing pearl millet mapping populations appear to offer opportunities for mapping pearl millet stover-quality-related traits as their parents differ substantially in combining ability for these traits (Table 1):

- ICMB 841 × 863B: medium duration, photoperiod-insensitive, tall elite × tall elite.
- PT 732B × P 1449-2: long duration, photoperiod-sensitive, dwarf elite × very tall germplasm.

Initial results have been obtained from QTL mapping of stover yield and quality parameters using the (ICMB 841 × 863B)-based population revealed several stover yield QTLs, but none appear to be independent of flowering time and/or plant height QTLs that are normally be expected to be associated with stover, grain, and biomass yield (Table 2). However, it has proven difficult to detect statistically significant QTLs for in vitro measurements associated with ruminant nutritional quality. Some reasons for this

Table 1

General combining abilities (GCAs) for leaf blade gas (ml/200 mg incubated dry matter) over a 96 h incubation period, for stover samples collected from testcross hybrids of 18 pearl millet mapping population parental lines and two elite forage pollinator line controls crossed to four genetically, cytoplasmically, and phenotypically diverse male-sterile lines, rainy season 1999, ICRISAT, Patancheru, India^a

	Incubation period (h)									
	3	6	9	12	18	24	36	48	72	96
Minimum	<i>-0.29</i>	<i>-0.60</i>	<i>-0.95</i>	<i>-1.01</i>	<i>-1.00</i>	<i>-1.89</i>	<i>-1.34</i>	<i>-1.63</i>	<i>-1.05</i>	<i>-1.29</i>
Maximum	+0.53	+0.68	+0.97	+1.00	+1.20	+1.58	+2.43	+2.27	+0.92	+0.93
Selected mapping population parental line pairs										
PT 732B	+0.04	+0.02	+0.37	+0.60	+0.73	+0.61	+0.30	+0.33	-0.26	-0.80
P 1449-2	+0.29	+0.68	+0.96	+1.00	+1.04	+1.58	+2.43	+2.27	+0.92	+0.88
ICMB 841	+0.53	+0.65	+0.97	+0.72	+0.57	+1.35	+1.82	+1.96	+0.87	+0.93
863B	+0.20	+0.25	+0.41	+0.23	-0.17	+0.26	+0.76	+1.02	+0.63	+0.67

^a Maximum observed GCA values for a particular incubation time, across all pearl millet mapping population parental line testcrosses are given in bold font, while minimum observed GCA values are given in italics.

include the relatively low operational heritabilities of the *in vitro* measurements (due to part of the *in vitro* measurements have been conducted on only a portion of the available field replicates) and due to the small numbers of mapping population progenies (79) used in the field trial. The best putative QTL initially detected for *in vitro* measures has been for gas production from the leaf blade fraction of the stover, which maps to the top of LG7 of 863B, and accounts for ca. 20% of the observed variation for this trait in mapping population progeny hybrids produced with only one of the two testers. In order to identify more QTLs for stover quality traits it will probably be necessary to move over to NIRS-based predictions of quality component traits from scans of ground stover samples (in order to assess all samples from all field replicates), and to use larger numbers of mapping population progenies in order to allow detection of statistically significant QTLs that account for more modest portions of the observed phenotypic variation. This increase in effective mapping population size will probably require that we use only one tester.

Initial attempts at MAS to improve stover yield and quality in pearl millet are now underway at ICRISAT, Patancheru, India for ruminant nutritional quality of stover and for straw yield.

For ruminant nutritional quality of stover fractions: firstly, marker-assisted backcross transfer of the putative leaf blade gas QTL from LG7 of 863B to ICMB 841 is underway and has now advanced to BC₄F₁, and near-isogenic versions of ICMB 841 with 863B alleles

in this genomic region should be available for testing within 18 months; secondly, near-isogenic populations have been developed by random-mating groups of mapping population progenies homozygous for ICMB 841 or for 863B alleles at markers flanking the QTL and assessment of hybrid performance of these two near-isogenic populations is underway.

The target of this study is early access to large straw samples for *in vivo* feeding trials that can be used to assess the utility of this putative QTL in terms of livestock performance. This approach was initiated at a time before we had access to facilities at ICRISAT, Patancheru for NIRS and small ruminant *in vivo* stover quality assessment, in order to ascertain whether, in fact, the allele from 863B at the putative QTL for increased gas production from the leaf blade fraction would have a favorable or unfavorable effect on animal performance. It was considered less expensive and time consuming to conduct this simple selection experiment than to await authorization to purchase equipment for NIRS or develop facilities for replicated large ruminant *in vivo* assessment of stover quality.

For straw yield (via increased plant height) and disease resistance (downy mildew and rust related to both yield and quality): QTLs have been backcrossed from LG1 of ICMP 451 to H 77/833-2 (Fig. 1), and yield assessment of their “improved” hybrids is underway. Initial results suggest a 10% stover yield advantage of the hybrids of the best of these introgression lines over those of their near-isogenic recurrent parent H 77/833-2.

Table 2

Significant QTLs identified (using MapMaker/QTL) following QTL analysis of 36 phenotypic traits for mapping F₃ testcross progeny data for the (ICMB 841 × 863B)-derived pearl millet mapping population evaluated in the 1998 summer drought nursery (fully irrigated control treatment) at ICRISAT, Patancheru^a

Trait ^b (mean, variance, skewness)	QTL detected							
	Linkage group	Position	Genetics	Weight	Dominance	Variation explained (%)	LOD	Proposed QTL names
HBloom (+43.04, +1.53, -0.03)	LG5	M345 + 02.4	Additive	-1.226	+0.000	28.7	5.02	
	LG7	M269 + 09.0	Additive	-1.084	+0.000	21.0	2.65	
	LG5	M345 + 03.6	Additive	-1.150	+0.000	45.8	7.88	QDtf.icp-LG5.1
	LG7	M269 + 09.8	Additive	-1.005	+0.000			QDtf.icp-LG7.1
HStDWm (+376.71, +30.75, +0.27)	LG3	M325 + 00.0	Additive	+14.432	+0.000	10.7	1.76	
	LG5	M345 + 00.5	Additive	-19.896	+0.000	18.8	2.97	
	LG5	M735 + 11.3	Additive	-17.307	+0.000	15.6	2.54	
	LG3	M325 + 00.1	Additive	+13.663	+0.000	28.2	4.98	QSdw.icp-LG3.1
	LG5	M345 + 00.3	Additive	-19.198	+0.000			QSdw.icp-LG5.1
MBHGas36 (+25.34, +5.33, +0.33)	LG7	M718 + 04.0	Additive	+3.912	+0.000	22.8	2.70	QBg3.ilr-LG7.1
PBloom (+43.09, +1.28, -0.75)	LG5	M345 + 04.4	Additive	-0.703	+0.000	13.4	2.05	
	LG6	M588 + 09.0	Additive	-0.911	+0.000	18.1	2.78	
	LG6	M870 + 15.8	Additive	-0.863	+0.000	17.2	2.23	
	LG7	M269 + 13.9	Additive	-0.887	+0.000	22.6	3.66	
	LG5	M345 + 07.0	Additive	-0.722	+0.000	46.6	8.88	QDtf.icp-LG5.1
	LG6	M588 + 12.8	Additive	-0.710	+0.000			QDtf.icp-LG6.1
PStDWm (+324.53, +27.58, +0.18)	LG7	M618 + 14.1	Additive	-0.801	+0.000			QDtf.icp-LG7.2
	LG5	M345 + 03.2	Additive	-15.744	+0.000	14.7	2.22	
	LG6	M514 + 01.0	Additive	-24.542	+0.000	27.4	4.51	
	LG6	M870 + 17.8	Additive	-20.870	+0.000	22.1	2.88	
	LG7	M618 + 04.5	Additive	-18.613	+0.000	19.5	2.66	
	LG5	M345 + 05.1	Additive	-14.690	+0.000	49.3	8.53	QSdw.icp-LG5.1
	LG6	M588 + 09.2	Additive	-19.330	+0.000			QSdw.icp-LG6.1
LG7	M618 + 07.6	Additive	-13.951	+0.000			QSdw.icp-LG7.1	

^a Statistically significant single or multiple-QTL models explaining the maximum portion of observed phenotypic variation for a particular trait are given in bold font.

^b HBloom: mean 50% bloom date (days after sowing) of H 77/833-2 testcrosses. HStDWm: mean stover dry weight per square meter (g) of H 77/833-2 testcrosses. MBHGas36: mean gas production (ml) from leaf blade samples of H 77/833-2 testcrosses incubated for 36 h. PBloom: mean 50% bloom date (days after sowing) of PPMI 301 testcrosses. PStDWm: mean stover dry weight per square meter (g) of PPMI 301 testcrosses.

4. Opportunities to exploit existing QTL information to improve stover quality in sorghum

The stay-green trait is the best-characterized component of post-flowering (i.e., terminal) drought tolerance available in sorghum (Walulu et al., 1994; van Oosterom et al., 1996; Borrell et al. 1999, 2000a,b; Borrell and Hammer, 2000; Thomas and Howarth, 2000; Xu et al., 2000a; Mahalakshmi and Bidinger,

2002; Sanchez et al., 2002; see also Thomas and Zerbini, 2003). Stay-green is also associated with resistance to charcoal stalk rot (Tenkouano et al., 1993) and superior ruminant nutritional quality of grain crop residues under conditions of terminal drought stress. It appears to result from altered nitrogen uptake and utilization that reduces hastening of leaf senescence under conditions of terminal drought stress (Borrell and Hammer, 2000). The stay-green trait enhances grain yield under stress by maintaining

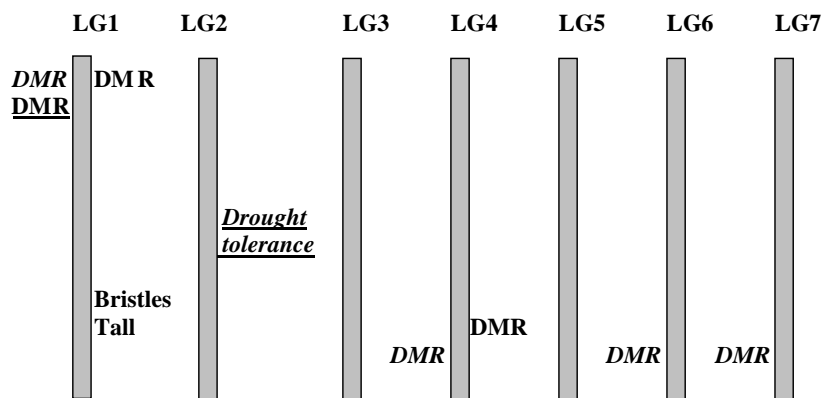


Fig. 1. Simplified schematic diagram of genomic distribution of QTLs for downy mildew resistance (DMR), terminal drought tolerance, and enhanced straw yield (associated with tall plant height linked to a major gene for long panicle bristles) being used for maintenance and improvement of popular, early-maturing dual-purpose pearl millet hybrid HHB 67 = 843A × H 77/833-2. Trait sources for improvement of 843A/B (to the left of each linkage group, LG): *ICMP 85410*, and *P7-3*; trait sources for improvement of H 77/833-2 (to the right of each linkage group): selection of *ICMP 451* and *PRLT 2/89-33*.

photosynthetically active green leaf area, and is also expected to result in more nutritive stover, under drought stress conditions—when livestock feed resources are typically most limiting. Breeding line B35 is the best-characterized source of the stay-green component of terminal drought tolerance available in sorghum. Other well-documented sources include E 36-1 and SC 56. Putative QTLs for the stay-green trait from B35 have been identified in five recently published studies (Tuinstra et al., 1997; Crasta et al., 1999; Xu et al., 2000b; Tao et al., 2000; Subudhi et al., 2000) based on four different mapping populations, and several of these have also been detected in an RIL population based on stay-green donor SC 56 (Kebede et al., 2001). QTL mapping of stay-green in two RIL populations based on stay-green donor E 36-1 has recently been completed as a planned by-product of a German-funded ICRISAT project to map *Striga* resistance (Haussmann et al., 2002b). Further, some 220 polymorphic sequence-tagged microsatellite (STMS) or simple sequence repeat (SSR) marker loci (Brown et al., 1996; Taramino et al., 1997; Kong et al., 2000; Tao et al., 2000; Bhatramakki et al., 2000; Klein et al., 2001; Schloss et al., 2002) have recently been added to the RFLP-based genetic linkage map of sorghum (Peng et al., 1999; Haussmann et al., 2002a). These SSR marker loci are highly polymorphic and suitable for use in a backcross program in which selection is based on high-throughput DNA-marker genotyping.

An integrated interactive sorghum linkage map, incorporating information on RFLP and SSR marker locus positions as well as approximate map positions of stay-green QTLs based on all available published information has been developed at ICRISAT, Patancheru, and is being made available globally via the ICRISAT website.

In addition, it should be practical to improve ruminant nutritional quality of sorghum stover by MAS for improved foliar leaf disease resistance (Klein et al., 2001), especially to diseases that reduce effective photosynthetic area under more favorable, higher rainfall conditions that favor fungal pathogens causing leaf blight and anthracnose. Such a breeding program should improve sorghum grain and stover yield by maintaining photosynthetically active leaf area, and would be expected to result in more nutritive stover that is not infested with fungi—having higher nutritive content and lower levels of fungal anti-metabolites. As shown by Pande et al. (2003), plant diseases can have serious negative effects on stover quantity and quality.

5. Marker-assisted introgression of the stay-green trait into tropically adapted, farmer-accepted dual-purpose (grain + stover) sorghums

Backcrossing of the stay-green trait into tropically adapted sorghum cultivars was initiated at ICRISAT,

Table 3

Sorghum recurrent parents for which marker-assisted backcrossing has been initiated at ICRISAT, Patancheru, India to introgress gene blocks controlling the stay-green component of terminal drought tolerance from donors B35 and E 36-1

Recurrent parents	Origin	Donors	Most advanced generation
296B	India	B35 and E 36-1	BC ₃ F ₁ and BC ₂ F ₁
ICSV 111	ICRISAT	B35 and E 36-1	BC ₃ F ₁ and F ₁
ICSV 112	ICRISAT	B35 and E 36-1	BC ₃ F ₁ and BC ₂ F ₁
ICSV-LM 90501	ICRISAT–LASIP	B35 and E 36-1	BC ₃ F ₁ and BC ₂ F ₁
ISIAP Dorado	ICRISAT	B35 and E 36-1	BC ₄ F ₁ and F ₁
M 35-1	India	B35 and E 36-1	BC ₂ F ₁ and F ₁
Macia	ICRISAT	B35 and E 36-1	BC ₃ F ₁ and BC ₂ F ₁
R 16	India	B35 and E 36-1	BC ₄ F ₁ and F ₁
S 35	ICRISAT	B35 and E 36-1	BC ₃ F ₁ and BC ₂ F ₁
CSV 4 = CS 3541	India (converted <i>zera-zera</i>)	B35 and E 36-1	BC ₂ F ₁ and F ₁
CSV 8R	India	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
CSV 14R	India	B35 and E 36-1	BC ₂ F ₁ and F ₁
CSV 15	India	B35 and E 36-1	Crossing to be initiated
Framida	South Africa	B35 and E 36-1	BC ₂ F ₁ and F ₁
Godamhuman	Sudan	B35 and E 36-1	BC ₂ F ₁
HC 260	India	B35 and E 36-1	BC ₂ F ₁ and F ₁
ICSB 11	ICRISAT	B35 and E 36-1	BC ₂ F ₁ and F ₁
ICSB 101	ICRISAT	B35 and E 36-1	BC ₂ F ₁ and F ₁
ICSV 401	ICRISAT	B35 and E 36-1	BC ₂ F ₁ and F ₁
ICSV-LM 86513	ICRISAT–LASIP	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
ICSV-LM 89522	ICRISAT–LASIP	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
ICSV-LM 90541	ICRISAT–LASIP	B35	BC ₂ F ₁
IRAT 204	IRAT	B35	BC ₂ F ₁
Kat 83389	Kenya	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
LARSVYT	ICRISAT–SADC	B35 and E 36-1	BC ₂ F ₁ and F ₁
M 148	India	B35 and E 36-1	BC ₂ F ₁ and F ₁
Malisor 84-7	ICRISAT–Mali	B35 and E 36-1	BC ₂ F ₁ and F ₁
Nagawhite	Mali	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
Patancheru local	India	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
RS 29	India	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
SDSL 87046	ICRISAT–SADC	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
SDSL 87574	ICRISAT–SADC	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
SDSL 88928	ICRISAT–SADC	B35 and E 36-1	BC ₂ F ₁ and F ₁
Seredo	Eastern Africa	B35 and E 36-1	BC ₂ F ₁ and F ₁
SPV 422	ICRISAT	B35 and E 36-1	BC ₂ F ₁ and F ₁
SPV 467	India	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
SSG 59-2	India	B35 and E 36-1	BC ₂ F ₁ and F ₁
Swathi	India	B35 and E 36-1	BC ₂ F ₁ and BC ₁ F ₁
Madau 131	Indonesia	B35 and E 36-1	F ₁ and F ₁
UPCAS 1-132	Indonesia	B35 and E 36-1	F ₁ and F ₁
Keris	Thailand	B35 and E 36-1	F ₁ and F ₁
Uthong 1	Thailand	B35 and E 36-1	F ₁ and F ₁
Suphanbur 11	Thailand	B35 and E 36-1	F ₁ and F ₁
Suphanbur 160	Thailand	E 36-1	F ₁
LS 1	PR China	B35 and E 36-1	F ₁ and F ₁
LS 2	PR China	B35 and E 36-1	F ₁ and F ₁
LS 3	PR China	B35 and E 36-1	F ₁ and F ₁

Patancheru before QTL mapping was completed, using donors B35 and E 36-1, and recurrent parents of interest to crop–livestock production systems in Africa, Asia and Latin America. MAS was initiated with a subset of the recurrent parents in 2001 (Table 3), and we are now seeking support to expand this program.

We have recently completed advancing nine recurrent parents (widely released improved open-pollinated variety (OPV) *ICSV 112*; elite hybrid seed parent maintainer *296B* and *rabi* (post-rainy season)-adapted OPVs M 35-1 and R 16 from India, as well as improved OPVs *ICSV 111*, *Macia*, and *S 35* from Africa, and *ICSV-LM 90501*, and *ISIAP Dorado* from Latin America) from BC₂ to BC₃ for stay-green donor B35 using SSR-based MAS targeting six stay-green QTLs detected in three or more of the published studies for this trait. We have also advanced five of these recurrent parents (given in italics above) from BC₁ to BC₂ for stay-green donor E 36-1. Finally, in case of recurrent parents *ISIAP Dorado* and R 16, we are currently advancing from BC₃F₁ to BC₄F₁ with introgression of donor segments flanked by SSR markers for single and multiple stay-green QTLs from donor B35 in order to identify which QTL or combination of QTLs can provide the greatest phenotypic improvement per unit operational expenditure in an applied sorghum drought tolerance backcrossing program. Our current funding will only permit continuation with introgression of stay-green QTLs from donor B35 to *ISIAP Dorado* and *rabi*-adapted fully senescent Indian breeding line R 16.

The first finished products from this marker-assisted backcrossing program (in the genetic background of drought-sensitive, three-dwarf, tan plant, white grain, tropically adapted, elite OPV *ISIAP Dorado*, which has large, hard grains and >10 t ha⁻¹ grain yield potential and has been released/cultivated in Mexico, El Salvador, Paraguay and Egypt) should be identified at ICRISAT, Patancheru by mid-2003 and available for phenotypic assessment of its grain and stover yield and quality by the post-rainy season (September/October sowing dates) of 2003/2004. These finished products should include single-QTL substitution lines for *Stg1*, *Stg2*, *Stg3* and *Stg4*, and one additional putative stay-green QTL each on sorghum linkage groups A and B, and as well as several multiple-QTL substitution lines based on donor B35.

6. Conclusions

6.1. Pearl millet

- Stover yield appears easier to assess and improve than does stover quality, at least if quality assessment is limited to in vitro estimates using many small samples.
- Genetic variability exists within cultivated pearl millet germplasm for assessed parameters, but the relevance of this in vivo has yet to be determined.
- MAS is possible in pearl millet with both RFLPs and SSRs:
 - Within mapping populations for earlier in vivo assessment.
 - Backcrossing traits into elite hybrid parental lines for earlier on-farm adoption opportunities.

6.2. Sorghum

- Stover quality parameters are not yet mapped, but several opportunities exist for marker-assisted breeding for traits expected to be related to nutritional value of stover for ruminant livestock. These include:
 - Marker-assisted backcross introgression of the complex stay-green trait using SSRs (this is underway and ICRISAT will soon have near-isogenic materials available for in vivo assessment).
 - Conventional and marker-assisted breeding for foliar disease resistance for dual-purpose sorghum improvement, or indeed for improvement of the nutritional value of residues for any crop in which these are used as feedstuffs for ruminant livestock.

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