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Pearl millet (*Pennisetum glaucum*) restorer lines for breeding dual-purpose hybrids adapted to arid environments

O P YADAV[1], K N RAI[2], F R BIDINGER[3], S K GUPTA[4], B S RAJPUROHIT[5] and S K BHATNAGAR[6]

All India Coordinated Pearl Millet Improvement Project, Mandor, Jodhpur 342004

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ABSTRACT

The adoption of pearl millet hybrids in the north-western arid zone has been very limited in contrast to their widespread coverage in relatively more favourable areas. This research was conducted to assess the combining ability of progenies derived from a composite, called Mandor Restorer Composite (MRC) that was synthesized using 12 lines with inbuilt combination of characters that are useful for arid environments. Testcross hybrids of 43 MRC progenies made on five diverse male-sterile lines were evaluated for their performance in four environments over three years (2005–07) under rainfed conditions of arid zone. There existed significant and exploitable differences among restorer (R) lines in their combining ability for biomass, grain yield, stover yield, harvest index and panicle harvest index under receding moisture conditions. General combining ability (GCA) for early flowering was consistently and positively related to GCA for grain yield. GCA for harvest index was positively and significantly related to GCA for grain yield, but was variably negatively related to stover yield. Neither GCA for HI nor GCA for time to flowering had as large an effect on GCA for grain and stover yields as did GCA for biomass. The results suggested that GCA for earliness, biomass and HI are not necessarily mutually antagonistic characteristics under arid zone conditions indicating that there are good prospects of identifying lines to produce hybrids with enhanced grain and stover yields without compromising crop duration. This study also identified a few progenies that had significant GCA for grain and stover yield in early-maturity background.

Key words: Adaptation, Arid, Biomass, Breeding, Drought, Harvest index, Pearl millet

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important cereal in the arid region of north-western India and is valued for both grain and stover. This region accounts for approximately 40% of the country's total area of about 9 million ha under pearl millet. The crop yield in this region is much lower than that in other regions because of adverse climatic conditions (Yadav *et al.* 2011) and limited adoption of improved cultivars, especially hybrids, in contrast to their widespread adoption in relatively more favourable pearl millet growing areas receiving more than 400 mm seasonal rainfall and having better soil fertility (Khairwal and Yadav 2005).

The arid zone is characterized by severe moisture stress. Germplasm from more favourable zones (on which most current hybrids are based) appears to be less adapted to such stress than is germplasm from this zone itself (Yadav 2010, Bidinger *et al.* 2006, Presterl and Weltzien 2003). During extreme drought stress years, farmers also perceive greater risk of crop failure from hybrid cultivation that had not been specifically bred for adaptation to this zone as compared to local landraces; and this is one of the main reasons for low adoption of current hybrids (Kelley *et al.* 1996). Yet hybrids, *per se*, still remain the best opportunity for raising grain and stover yields in the arid zone (Yadav *et al.* 2011). Hence there is a need to breed hybrids that are as well adapted to the arid zone as the indigenous landrace varieties.

Pearl millet improvement for the arid zone of north-western India has not been a priority research area, till recent past, on the agenda of the Indian Council of Agricultural Research (ICAR) as well as the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) (Bidinger *et al.* 2009). In the recent years under ICAR-ICRISAT partnership research programme, however, there has been relatively greater emphasis than in the past to breed hybrid parental lines better adapted to the arid zone, and thus to help develop hybrids adapted to this region. One of the approaches followed in this attempt is to constitute and improve populations based on lines developed at the pearl millet improvement centres in this zone and Mandor Restorer

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[1] Project Coordinator (e mail: opyadav21@yahoo.com), [2] Principal Scientist (e mail: k.rai@cgiar.org), [3] Principal Scientist (deceased), [4] Scientist (e mail: s.gupta@cgiar.org), [5] Associate Professor (e mail: balwantraj@rediffmail.com), [6] Former Project Coordinator (e mail: bhatnagarsk@rediffmail.com)

Composite (MRC) is one example of such populations. This paper reports on the evaluation of ability of advance progenies derived from this composite to develop dual-purpose hybrids adapted to arid environments.

MATERIALS AND METHODS

As a part of the ICAR-ICRISAT research partnership, 12 diverse restorer lines developed at the AICPMIP Centers, Mandor (4); CCS Haryana Agricultural University, Hisar (3); Gujarat Agricultural University, Millet Research Station, Jamnagar (3); and Rajasthan Agricultural University, Agricultural Research Station, Jaipur (2) were intercrossed in a diallel fashion to develop 66 hybrids during the 1999 summer season at ICRISAT, Patancheru. These hybrids were subjected to two rounds of random mating to constitute MRC.

During the final random mating in the early 2000 summer season, open-pollinated panicles of 235 selected plants were harvested as half-sibs. These were evaluated in an unreplicated nursery at ICRISAT during the 2000 rainy season. Based on visually assessed agronomic performance, 93 half-sibs were selected. Using the remnant seed, these half sibs were replanted during the 2001 summer season and 245 S1 progenies were produced from 81 selected half-sibs. This was followed by further selection for agronomic performance in the rainy and summer season breeding nurseries. In another breeding stream, The MRC bulk was planted during the 2001 summer season and 476 S_1 progenies were produced. These were further subjected to inbreeding and selection based on visually assessed agronomic performance.

At each breeding stage, selection laid greater emphasis on earliness. During the course of inbreeding and selection, the progenies were evaluated and selections made for resistance to downy mildew, using green house seedling screening techniques against Durgapura pathotype at the S_2, S_3 stages of inbreeding. At the S_4 stage, evaluation for resistance was done both for Durgapura and Jodhpur pathotypes. At each screening stage, progenies generally showing less than 10% downy mildew incidence were selected. Finally 43 lines derived from MRC were selected for this study.

The selected 43 MRC progenies (plus five inbred checks) were test crossed to five elite male-sterile (A) lines, viz. HMS 7A, ICMA 91 444, ICMA 95 111, ICMA 98 111 and ICMA 99022 considered to be reasonably well-adapted to the arid zone (Yadav *et al.* 2003). The checks were a combination of restorers (ICMR 01004 and ICMR 356) of hybrids recommended for the arid zone and restorer lines and populations based on arid zone landraces (Early Rajasthan and IP 3 228 Restorer Populations and an S_6 line from the Barmer Restorer Population). The resulting 240 testcrosses were evaluated in replicated trials in two arid zone locations (RAU Nagaur and CAZRI Jodhpur between 2005 and 2007).

The plots were over sown with a tractor-drawn planter and stand thinned to approximately 6 plants/m^2 (60 000 plants/ha). 20 kg N/ha and 20 kg P_2O_5/ha were banded into the ridges before sowing and 23 kg N/ha was side dressed at approximately three weeks after emergence. Weeds were controlled by hand weeding. Trials were well-managed, but were grown under entirely rainfed conditions; because of this, two of the trials planted (Nagaur 2006 and CAZRI 2005) had to be abandoned due to severe stress.

Because of the size of the experiment, plots were restricted to single rows, in a 10 (plots/block) by 24 (blocks per replication) alpha design, with three replications. General combining ability (GCA) estimates for each line in each trial were based on the mean of 15 values (5 testers × 3 reps), which partly overcame additional variability of single row plots. Across environments, the GCA values for R lines were based on a mean of 60 plots.

At maturity, panicles were harvested, counted and sun dried for about a week. Panicles were then weighed, mechanically threshed and the grain weighed immediately. Stover was cut at ground level, bundled and left in the field to dry for about two weeks and weighed. These data were used to calculate biomass, grain and stover yields on a square meter basis, and harvest index (HI), and panicle harvest index (PNHI) as an estimate of success in setting and filling grains under terminal stress on a percentage basis. Data were analyzed by the Genstat REML programme, according to the design, with A/B lines and R lines as fixed effect and replications and blocks within replications as random effects. The across environment analysis was done in the same fashion, with environment as a fixed effect. GCA estimates were calculated for biomass, HI, PNHI, grain and stover yields. Correlations among GCA values were estimated to better understand the basis of superior combining ability for grain and stover yield.

RESULTS AND DISCUSSION

Evaluation environments

Mean grain yields of the trials ranged from a low of 340 kg/ha in 2005 to a high of 1 520 kg/ha in 2007, which covers virtually the full range of expected grain yields in the arid zone, under fully rainfed conditions (Table 1). Mean yield across environments (952 kg/ha) was higher than farm yields in the arid zone, but considerably lower than average yields from experimental station sites. As drought stress is the predominant environmental feature of the arid zone, it is essential that restorer lines targeting this zone are evaluated under representative drought stress conditions.

The effects of both pre- and post-flowering drought stress were evident in most of the trials. Mean time to flowering ranged from 44 to 53 days, largely due to the occurrence and severity of pre-flowering stress, which was a factor in all locations apart from Jodhpur 2006, and which resulted in significant delay in flowering in both Nagaur

Table 1 Hybrid trial mean and probability levels for differences among the mean hybrid performance of R-lines

Variable	Nagaur 2005	Jodhpur 2006	Nagaur 2007	Jodhpur 2007	Across environ.
Seasonal rainfall (mm)	474	208	367	234	321
Trial means					
Flowering (d)	50.4	44.0	47.5	53.1	48.8
Biomass (g/m)	223	211	383	372	297
Harvest Index (%)	15.0	33.6	39.9	32.7	30.3
Panicle HI (%)	49.0	64.7	79.6	66.1	64.9
Grain yield (g/m)	34	72	152	123	95.2
Stover yield (g/m)	155	101	192	188	159
R line differences (probability level)					
Flowering	0.001	0.001	0.001	0.001	0.001
Biomass	0.001	0.001	0.02	0.001	0.001
Harvest index	0.001	0.001	0.001	0.001	0.001
Grain yield	0.001	0.001	0.006	0.001	0.001
Stover yield	0.001	0.001	0.04	0.001	0.001

2005 and Jodhpur 2007 (Table 1). Mean PNHI also indicated that all environments apart from Nagaur 2007 experienced post-flowering stress as well, as PNHI values are usually = 75% where grain filling is unaffected by stress. The 2005 Nagaur trial experienced very severe post-flowering stress (despite receiving the highest seasonal rainfall of all four experiments), as evidenced by the very low panicle harvest index (49%) and a harvest index values of only 15% (Table 1). Post-flowering stress affected both PNHI and HI, although to a lesser extent, in the two Jodhpur locations. The 2007 Nagaur trial was the only one to be free of severe stress prior to and after flowering, but early growth was still affected by a long post-sowing stress, which set a limit on biomass grain and stover yields, despite the high HI and PNHI values (Table 1).

Analysis of variance indicated that there were significant differences among R-lines for their mean hybrid performance (a measure of combining ability) for all traits in all trials, as well as across environments (Table 1). Thus, the experiments were successful in identifying differences in adaptation among the MRC lines and checks to the arid zone, which can be exploited in breeding new hybrids for this zone.

Relationships among GCA's for various traits

Linear correlations were used to evaluate the importance of GCA for various measured traits as determinants of GCA for grain and stover yields. GCA for early flowering, which is widely believed to be an essential trait for the arid zone, was consistently and positively related to GCA for grain yield, although it accounted for <15% of the variation in GCA for grain yield across environments (Table 2). GCA for earliness was significantly related to GCA for HI across environments, as is generally the case for pearl millet, but generally unrelated to GCA for biomass or stover productivity. As a consequence, early flowering lines tend to favour grain production over stover production, and thus may be of less interest to farmers who depend on the crop for both stover for their ruminants and grain for family food, than to those interested only in the grain.

Differences in combining ability for biomass productivity was the overwhelming determinant of differences in combining ability for both grain (r = 0.82***, $P<0.001$) and stover (r = 0.88***, $P<0.001$) productivity among the MRC lines across environments (Table 2). This is consistent with findings from earlier experiments (Bidinger *et al.* 2003), and with our proposed measure of adaptation of the arid zone (Bidinger *et al.* 2006). The finding that the ability to produce biomass under arid zone conditions is also the single most important trait determining yield in breeding lines suggests that biomass productivity in arid zone environments proved a functional measure of "adaptation", which is a rather difficult trait to measure/use in a breeding programme. The relationship between GCA for biomass and GCA for grain and stover

Table 2 Correlations of GCA for earliness, biomass, harvest index, panicle harvest index and grain and stover yields in the MRC lines

	Nagaur 2005	Jodhpur 2006	Nagaur 2007	Jodhpur 2007	Across environ.
Earliness vs biomass	–0.01	+0.27	+0.32*	–0.08	+0.04
Earliness vs HI	+0.51**	+0.82**	+0.04	+0.62**	+0.64**
Earliness vs PNHI	+0.32*	+0.59**	-0.07	+0.61**	+0.38**
Earliness vs grain	+0.32*	+0.62**	+0.34*	+0.35*	+0.38**
Earliness vs stover	–0.28	–0.20	+0.27	–0.26	–0.22
Biomass vs HI	+.28	+.28	+.03	-0.11	+0.08
Biomass vs grain	+.77**	+.84**	+.93**	+0.67**	+0.82**
Biomass vs stover	+.93**	+.78**	+.93**	+0.87**	+0.88**
HI vs grain yield	+0.81**	+0.74**	+0.38**	+0.66**	+0.61**
HI vs stover yield	–0.03	–0.32*	–0.25	–0.52**	–0.34
PNHI vs grain yield	+0.67**	+0.56**	+.07	+0.53**	+0.35*

*, ** significant at 0.05 and 0.01 levels of probability

Table 3 GCA estimates of the MRC lines and checks for a range of traits, based on five testers and four trial environments

MRC line	Days to flower†	Biomass yield (g/m)	Harvest index (%)	Grain yield (g/m)	Stover yield (g/m)	Panicle HI (%)
Barmer Restorer Pop S_6 line	0.30	32.1**	–5.38	–8.6	40.6**	–1.36
Early Raj Restorer Pop	–1.05**	29.3**	1.34**	12.5**	16.9**	3.13**
ICMR 01004	–2.70**	–5.2	1.87**	5.1	–10.5	0.87
ICMR 356	–0.91**	–0.7	1.19**	4.5	–9.6	–1.58
IP 3228 Restorer Pop	–0.84**	10.7	0.92	2.7	7.2	1.78**
MRC HS-130–6-1-1-B-B-B	2.43	–32.5	–1.26	–12.3	–9.2	2.13**
MRC HS-142-2-6-2-B-B-B	–0.17	–34.1	0.61	–11.8	–13.6	1.81**
MRC HS-170-1-2-1-B-B-B	–2.17**	–7.5	2.32**	4.7	–9.7	2.18**
MRC HS-176-2-2-1-B-B-B	–0.23	9.0	–1.48	–2.4	4.4	–2.2
MRC HS-178-1-3-1-3-B-B	0.96	-28.9	–1.98	–12.8	–14.4	–3.58
MRC HS-183-1-1-3-B-2-B	–1.60**	–13.6	0.37	–2.8	–8.5	0.28
MRC HS-219-2-1-2-B-B	0.24	–29.8	1.38**	–7.6	–20.2	0.73
MRC HS-225-3-2-4-B-B-B-B	–1.53**	–30.1	2.75**	–4.3	–21.1	2.69**
MRC HS-225-3-5-2-B-B-B	1.91	2.7	–1.44	–6.2	6.7	–0.79
MRC HS-35-2-2-1-B-B-B	–0.64**	-13.6	1.67**	-1.9	-8.0	2.96**
MRC HS-41-2-2-4-B-B-B	0.52	17.4	1	9.3**	6.9	0.58
MRC HS-60-3-1-2-B-B-B	0.56	21.2**	–0.53	5.3	16.6**	0.73
MRC HS-64-1-2-1-B-1-B	–0.93**	–18.3	2.75**	–0.2	–15.8	2.12**
MRC HS-86-1-1-2-B-B-2	–2.82**	5.6	1.99**	6.1	0.4	3.18**
MRC HS-86-1-1-4-B-B-2	–2.53**	–0.6	0.71	1.5	–2.5	0.01
MRC HS-91-2-3-3-B-B-B	1.79	25.3**	–0.99	4.3	13.1**	–2.07
MRC HS-98-4-1-3-B-B-B	3.32	5.5	–4.34	–10.2	17.5**	–2.43
MRC HS-98-6-4-5-B-B-B-B	1.90	–22.5	–1.77	–12.5	–2.0	2.34**
MRC S1-107-1-3-B-B-B	–1.16**	50.1**	3.34**	26.1**	13.4**	0.81
MRC S1-109-1-1-1-B-B	1.89	–18.3	–2.85	–12.6	–4.0	–1.28
MRC S1-122-2-2-B-B-B	1.15	–4.5	–0.53	–1.6	–3.1	–1.46
MRC S1-155-4-2-B-B-B	–2.69**	–26.1	0.98	–3.8	–21.5	–1.24
MRC S1-155-4-3-B-B-B	–1.35**	12.3	0.93	7.3	6.8	2.18**
MRC S1-156-1-1-2-B-1-B	–0.23	–21	0.89	–4.1	–14.4	0.76
MRC S1-159-1-2-3-1-B	–0.45	10.9	–2.49	–4.1	12.3**	–2.69
MRC S1-168-1-2-B-B-B	1.95	–31.1	–1.44	–11.8	–17.8	–3.81
MRC S1-174-1-2-B-2-B	0.61	51.1**	–0.43	14.3**	30.3**	0.47
MRC S1-189-2-1-B-3-1	1.65	–14.7	–1.95	–9.8	–2.2	–1.44
MRC S1-202-4-2-2-2-B	1.31	–13.2	–0.4	–3.5	–7.6	–1.14
MRC S1-204-5-2-B-B-B-B	1.90	31.8**	0.48	11.8**	16.3**	0.2
MRC S1-214-2-5-B-B-B	1.13	7.4	–1.42	–0.9	11.2**	0.04
MRC S1-231-6-3-B-B-B	0.07	–15.4	–1.26	–8.5	–9.4	–2.34
MRC S1-353-3-2-B-B-B	0.80	16.0**	–0.67	3.7	12.0**	0.21
MRC S1-37-1-2-B-B-B	–0.09	–4.8	0.45	1.4	–4.6	0.14
MRC S1-385-3-2-B-B-B	0.11	–0.2	0.57	3.3	–5.6	–1.95
MRC S1-4-1-3-B-B-B	1.42	24.0**	0.62	10.3**	10.4	0.03
MRC S1-4-6-3-B-B-B-B	1.14	8.2	0.75	2.0	2.6	0.7
MRC S1-48-2-2-2-B-B	–0.57	16.3	1.30**	9.3	6.1	2.02**
MRC S1-61-1-1-B-B-B	–2.48**	12.8	–0.01	4.8	7.3	–0.17
MRC S1-62-1-1B-B-B	0.23	–22.3	–1.52	–10.7	–7.1	–1.11
MRC S1-66-1-5-B-B-B	–0.45	–29.0	0.06	–9.3**	–16.5	–1.1
MRC S1-85-2-2-B-B-B	–1.36**	50.3**	2.68**	24.9**	13.9**	0.42
MRC S1-89-1-1-B-B-B	–0.34	–14.4	0.25	–1.0	–13.9	–1.96
No. of lines with a significant ($P < 0.05$) positive gca	16	10	12	8	12	12

† GCA for early flowering has a negative value; ** significant and positive GCA at the $P < 0.05$ level

yields was also very consistent across the individual environments (Table 2) regardless of either the biomass or grain yield level achieved (Table 1).

GCA for HI was positively and significantly related to GCA for grain yield, but was variably negatively related to stover yield (Table 2). Manipulation of HI is relatively easy (due to its high heritability) and has been the classic route to increasing grain yield in all cereals (Austin *et al.* 1993; Sayre *et al.* 1997, Peng *et al.* 2000, Bidinger *et al.* 2003, Shastry 2006). For the arid zone, however, selecting for CGA for HI may be a questionable approach because of its negative effect on stover productivity (Yadav and Rai 2011) given that millet stover makes up more than 50% of the available feed for farm ruminants. Improved breeding material such as the MRC is likely to already have a good HI, so that selection can focus on improving biomass, and simply holding HI levels constant. There was no consistent or significant relationship between GCA for biomass and GCA for HI in this experiment (Table 2), so selection for improved biomass should be possible without an effect on HI. This argument may be less applicable to landrace-based materials, which generally have a higher GCA for biomass but a negative GCA for HI – the Barmer Restorer Population line (Table 3) is a classic example.

In three of the four environments, GCA for PHNI was significantly and positively related to GCA for grain yield; the exception was the Nagaur 2007, where there was adequate moisture for grain filling. Thus it appears that PNHI can be predictably used as a secondary selection criterion in environments where stress during grain filling affects grain yield. PNHI is a reflection of drought escape as well as tolerance of terminal drought (Table 2), so selection for GCA for PHNI should preferably be done within a common maturity group.

CGA of the MRC lines and checks

Of the total progenies, those that had significant CGA effects varied from 8 (17%) for grain yield to 16 (33%) for days to flower, suggesting a good level of genetic variability for GCA within the MRC (Table 3). Two of the checks (the Barmer line and the Early Raj Restorer Pop) and eight of the MRC lines had a significant positive GCA for biomass productivity. In six of these lines, this was accompanied by a significant positive GCA for grain yield (Table 3) and in five, by a positive GCA for both grain and stover yield. This is in agreement with the results of the correlations in Table 2 that a high GCA for biomass is linked to a high GCA for grain and stover yields. The four lines with a positive GCA for biomass but not for grain yield, all had a negative GCA for HI (Table 3). Consequently, all had a significant positive GCA for stover but not grain yield.

Three of the restorer lines (Early Raj Restorer Pop., MRC S1 107-1-3-B-B-B and MRC S1-85-2-2-B-B-B) actually had significant positive GCA for both biomass and HI, which was unexpected. This resulted, in the case of the MRC lines, in the two highest GCA values for grain yield, in addition to substantial GCA for stover yield (Table 3). These two MRC lines produced testcross hybrids with grain yields of 1 200–1 210 kg/ha, compared to the trial average of 952 kg ha^{-1} and to a grain yield of 1 000 kg/ha for ICMR 01004 hybrids (Table 3). Similarly, the stover yields of their testcrosses averaged 1 720–1 730 kg/ha, compared to the trial average of 1 590 kg/ha and to 1 480 kg/ha for ICMR 01004 hybrids (Table 3). The same three restorers also had significant positive GCA for earliness, as well as for biomass and HI, which again was unexpected. Clearly, the ability to produce biomass under arid zone conditions is not simply a consequence of a longer duration, as it mainly is in more favourable environments, but a reflection of a better ability to tolerate the low moisture/high temperature conditions of this zone.

It is also worth noting that the best of the MRC lines were clearly superior in combining ability to the two inbred restorer checks, ICMR 01004 (the restorer of the widely grown HHB 67-2) and ICMR 356 (the restorer of the recommended hybrid ICMH 356) both for grain and stover yields. Hence the MRC has the potential to produce new restorers that are superior to the pollinator lines of the commercial hybrids.

Implications in hybrid breeding for arid zone

The results of this experiment underlined the predominant role of the ability to produce biomass under arid zone conditions as the basis for improved grain and stover yields. Neither GCA for HI nor GCA for time to flowering (e g crop duration) had as large an effect on GCA for grain and stover yields as did GCA for biomass. The results of the experiment also suggest that GCA for earliness, biomass and HI are not necessarily mutually antagonistic characteristics under arid zone conditions. The best of the MRC lines (plus the Early Raj Restorer Pop) combined positive GCA values for all three traits, meaning that it is not necessary to sacrifice biomass for earliness or to sacrifice a high HI for a high biomass. If this holds in other materials, then selection of restorers that are good for all three traits will be much easier than would have been expected. Again, however, these traits can only be assessed in multi- environment combing ability trials.

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