Characterization of ICRISAT-bred restorer parents of pearl millet

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Introduction

Pearl millet (*Pennisetum glaucum*), primarily grown for grain production on more than 26 million ha in the arid and semi-arid tropical (SAT) regions of Asia and Africa, is a highly nutritious cereal crop with wide agroecological adaptation. India, the largest producer of this crop at global level, cultivates pearl millet on about >9 million ha contributing to more than 90% area of the crop in the Asian region. It is a highly cross pollinated crop, and single-cross hybrids generally give 20–30% more yield than open pollinated varieties (Rai et al. 2006). With the availability of commercially exploitable cytoplasmic-nuclear male sterility (CMS) systems in pearl millet, the national agricultural research system (NARS) and the private seed sector in India focused their breeding programs on hybrid development. This led to the development and adoption of a diverse range and large number of hybrids (>80 in 2011) and now occuping >4.5 million ha area, which is about half of the total pearl millet area being cultivated in India (Rai et al. 2006). The International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) also aligned its breeding program to develop promising hybrid parental lines for supporting Asian pearl millet hybrid program.

There has been extensive use of ICRISAT-bred seed parents (A/B lines) and restorers (R-lines) by both NARS and the seed companies in private sector. For instance, 60–70% of the hybrids often included in the All India Coordinated Trials and released by both NARS and the private sector are based on ICRISAT-bred hybrid parents (Mula et al. 2007). In view of the increased use of ICRISAT-bred parental lines and awareness on protecting these lines under Intellectual Property Rights (IPR), a need has been felt to characterize and place them in public domain as international public goods (IPGs). This would enhance ICRISAT's ability to protect these materials from any possible infringement, and allow unhindered free access to it by public organizations globally. Working in this direction, ICRISAT has already characterized and documented 99 A/B lines which were developed during the period 1981–2004 (Rai et al. 2009) (http://www.icrisat.org/what-we-do/publications/icrisat-publications-2010/morphological-pearlmillet.pdf).

The breeding program for the development of restorer parents runs parallel to the seed parent development program and about 1731 restorer lines were developed and designated during 1985–2008. Most of the restorers have been bred using genotypes of Indian and African origin, following several methods such as pedigree selection, population improvement or pedigree bulk selection in introduced landraces or breeding materials, followed by inbreeding at ICRISAT, and also few entries amongst them have been obtained from national program partners. These lines were designated and disseminated as potential hybrid parents after evaluation for agronomic performance and resistance to downy mildew (caused by *Sclerospora graminicola*), the most dreadful disease of pearl millet in India. Although individual lines were evaluated in the years they were developed and designated, they have not been evaluated for a comprehensive set of morphological traits in common environments until now. Thus, the objective of the present study was to characterize 114 promising restorer lines using 26 morphological traits developed as DUS (distinctness, uniformity and stability) descriptors (AICPMIP 2006).

Materials and methods

Plant material and field trials. In pearl millet improvement program at ICRISAT, about 1731 pearl millet restorers are maintained in the ICRISAT Pearl Millet Pollinator Collection (IPMPC). These were designated as IPC (ICRISAT Pollinator Collection) number in order of their development in the breeding program at ICRISAT. These restorers were bred at ICRISAT using ear to row method followed by their maintenance through bulking 10–15 phenotypically similar looking plants/season. All collection entries are being maintained in medium-term cold storage at

ICRISAT. Based on the information contained in a booklet "Pearl Millet Pollinators: Description, Pedigrees and Distribution" published by ICRISAT in 1995 (Talukdar et al. 1995) where 1704 restorers designated in the period 1985–95 have been documented, 114 restorers identified as relatively more promising based on plant characteristics and diversity were selected for this study. These 114 restorer parents were planted in a randomized complete block design with two replications during the 2007 rainy season (Jul–Oct) and 2008 postrainy (dry) season (Mar–May) at ICRISAT, Patancheru (18° N and 78° E). During the rainy season, total rainfall was 442.7 mm with the weekly mean maximum temperatures ranging between 28.1°C and 31.9°C, weekly mean minimum temperatures ranging between 17.4°C and 23.7°C and relative humidity above 96% (Fig. 1). During the postrainy season, total rainfall was 236.2 mm with the weekly mean maximum temperatures ranging between 28.8°C and 39.4°C, weekly mean minimum temperatures ranging between 14.6°C and 24.5°C and relative humidity >92% (Fig. 2).

Each line was machine-planted in single row of 4 m with 75 cm spacing between the rows in rainy season and 60 cm in postrainy season, and 15 cm plant spacing in both seasons. The experiment was conducted in Alfisols with applied fertilizer levels of 80 kg ha^{-1} N (18% basal and rest as top dressing) and 46 kg ha^{-1} as P (basal dose). The standard cultural and agronomic practices were followed that included thinning and manual weeding at 15 days after sowing.

Data collection and analysis. The observations on nine quantitative traits were taken on 10 random plants in each plot for plant height (cm), panicle length (cm), panicle diameter (cm), number of productive tillers, number of nodes per plant, leaf sheath length (cm), leaf blade length (cm), leaf blade width (cm) and 1000-grain weight (g). Time to 50% flowering was recorded on plot basis when the main panicles of 50% of the plants in the plots had full stigma emergence. Data were also taken on 16 other qualitative or quasi-qualitative traits. These included seedling color, panicle exertion, panicle tip sterility, node pubescence, node pigmentation, internode pigmentation, leaf sheath pubescence, anther color, glume pigmentation, presence/absence of bristles in panicle, and bristle color for which data were recorded on the basis of visual assessment of individual plants (or parts of plants) within a plot (VS); for traits such as plant growth habit, panicle shape, panicle density, seed color and seed shape it was based on visual assessment of group of plants (or parts of plants) in a plot (VG). The mean plot values of the quantitative traits measured were subjected to analysis of variance across the two seasons following randomized complete block design and using Genstat 10.1 software.

Results and discussion

Germplasm base. In the pearl millet improvement program at ICRISAT, pollinator lines (R-lines) are developed with considerable morphological and genetic diversity and then designated based on agronomic performance. The parentage of 114 designated restorer lines shows the utilization of a wide range of germplasm and improved lines in developing these R lines. For instance, 19 R-lines were directly selected from the germplasm accessions,

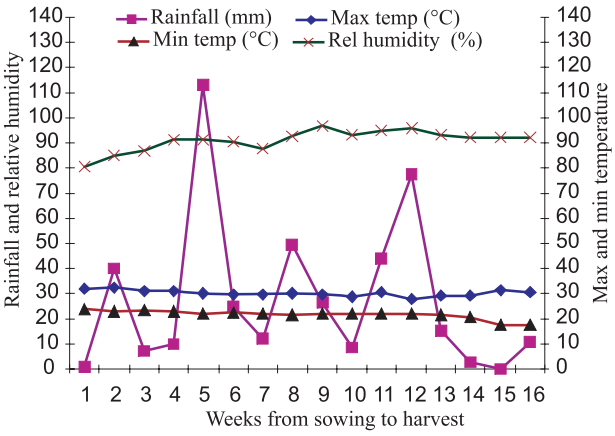

Figure 1. Weekly distribution of rainfall, maximum and minimum temperature and relative humidity during 2007 rainy season.

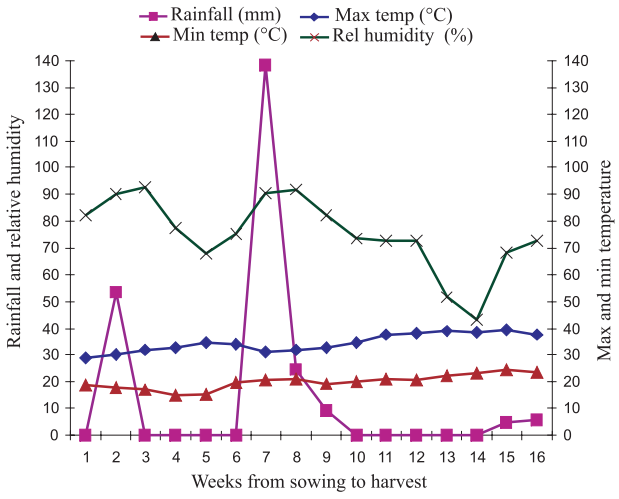

Figure 2. Weekly distribution of rainfall, maximum and minimum temperature and relative humidity during 2008 postrainy season.

and 11 were selected directly from composites (Table 1). In addition, 8 R-lines were derived from crosses that involved germplasm in their parentage and 17 lines had composites in their parentage. Thus, there were 55 restorer lines that had some components of germplasm and/or composite in their parentage, indicating apparently substantial usage of germplasm and composites in the development of these R-lines. The remaining 59 R-lines were derived from crosses between elite inbred lines. Thus, while these 114 restorer lines have been developed utilizing diverse parents, they also share a large number of parental lines of common origin. For instance, 43 restorer lines had one of the parents developed from Jamnagar Research Station (depicted with 'J' prefix in the pedigree), while 32 pollinator lines had one of the parents from Institute of Agricultural Research Millet Program, Kano, Nigeria (depicted as '700..' series as prefix) in their parentage. B282, a d_2 dwarf restorer obtained from Rockefeller Foundation Collection, originating from Bangkok, Thailand was found in the parentage of about 14 restorer lines. Again, LCSN, a selection from ICRISAT Late Composite developed in Burkina Faso was present in the parentage of about 14 lines.

Quantitative traits. The overall mean of all the R-lines for ten quantitative characters revealed significant effect of season on plant height (148 cm in rainy season; 134 cm in postrainy season), time to 50% flowering (48 days in rainy season; 50 days in postrainy season) and for number of nodes per plant (8.9 in rainy season; 8.3 in postrainy season) (Table 2). There were significant differences among genotypes for all the quantitative traits under study which revealed presence of significant genetic variability among the restorer lines. Wide range was observed for most of the characters based on the mean values over the two seasons (Table 3). For instance, plant height ranged from 56 to 201 cm, 50% flowering from 43 to 61 days, panicle length from 10 to 40 cm and 1000-grain weight from 5.7 to 14.0 g. There were 6 genotypes with more than 3 tillers per plant while 5 genotypes had panicle diameter of more than 3.0 cm. Restorer lines like ICMP 451 (IPC 107) and H77/833-2 (IPC 1466) which have been quite popular in pearl millet hybrid breeding programs were used as reference lines to classify the restorer lines for some specific traits. Twenty-one restorer lines were in the same maturity group as the earliest flowering commercial restorer parent H77/833-2 (45 days). About 47 R-lines were in the same productive tiller group as of ICMP 451 (1.8 tillers per plant). Maximum number of 27 R-lines was in the same panicle length group as of ICMP 451 (23 cm), while 11 lines had panicle length of more than 25 cm. There were 26 R-lines with similar panicle diameter as of ICMP 451 (2.8 cm) while 5 lines had more than 3.0 cm of panicle diameter. The 1000-grain weight of 21 lines ranged from 10.1 to 12 g as that of ICMP 451 (11.1g).

Table 1. Genetic diversification of 114 designated restorer parents of pearl millet at ICRISAT.

Germplasm base	No. of lines	Remarks	Genetic base (Code)
Germplasm	19	Inbreeding and selection directly from germplasm	1
Composites	11	Includes composites and open pollinated varieties	2
Germplasm × Elite line crosses	8	Includes early generation breeding lines derived from germplasm	3
Composite × Elite line crosses	17	Includes early generation breeding lines derived from composites	4
Elite line × Elite line crosses	59	Includes crosses between advanced generation lines	5

Table 2. Analysis of variance for 10 quantitative traits in pearl millet restorer lines evaluated at ICRISAT, Patancheru (2007 rainy season and 2008 postrainy season).

		Mean square[1]									
Source of variation	df	TF	LSL	LBL	LBW	PL	PD	NNP	NPT	PHT	TGW
Season	1	466.7**	1.7NS	59.4NS	0.6NS	1.7NS	0.84NS	41.9*	0.13NS	19373.4*	2.1NS
Replication/Season	2	3	1	109.3	1.3	11.9	0.14	1.4	1.2	693.4	1.6
Genotype	113	48.7**	12.7**	165.3**	1.1**	76.6**	0.65**	2.1**	1.1**	1930.3**	15.3**
Genotype × Season	113	11.2**	0.5**	20.3**	0.1**	2.2**	0.04*	0.5**	0.34**	179.1**	1.1**
Error	226	0.9	0.21	4.2	0.1	1.0	0.03	0.1	0.1	29.5	0.5

1. TF = Time to 50% flowering; LSL = Leaf sheath length; LBL = Leaf blade length; LBW = Leaf blade width; PL = Panicle length; PD = Panicle diameter; NNP = Number of nodes per plant; NPT = Number of productive tillers per plant; PHT = Plant height; TGW = 1000-grain weight. * = Significant at 5% level; ** = Significant at 1% level; NS = Not significant.

Table 3. Frequency distribution of designated pearl millet restorer lines for agronomic traits evaluated at ICRISAT, Patancheru (2007 rainy season and 2008 postrainy season).

Character		No. of R-lines in trait classes									Range	Reference R-line	
Plant height (cm)	Trait class	<90	90.1–100	100.1–110	110.1–120	120.1–130	130.1–140	140.1–150	150.1–160	>160	56–201	ICMP 451	149.8
	No. of lines	1	3	4	6	19	25	19	19	20			
Time to 50% flowering (days)	Trait class	<42	42.1–45	45.1–48	48.1–51	51.1–54	54.1–57	57.1–60	>60	–	43–61	H77/833-2	45
	No. of lines	–	21	31	35	20	8	1	–	–			
Number of productive tillers plant^{-1}	Trait class	1	1.1–2	2.1–3	3.1–4	4.1–5	5.1–6	>6	–	–	1.1–4.4	ICMP 451	1.8
	No. of lines	–	47	63	5	1	–	–	–	–			
Panicle length (cm)	Trait class	<15	15.1–20	20.1–25	25.1–30	30.1–35	>35	–	–	–	10.3–40.1	ICMP 451	22.9
	No. of lines	9	68	28	10	–	1	–	–	–			
Panicle diameter (cm)	Trait class	<2.0	2.0–2.5	2.6–3.0	3.1–3.5	3.6–4.0	–	–	–	–	1.6–3.8	ICMP 451	2.8
	No. of lines	21	63	27	4	1	–	–	–	–			
1000-grain weight (g)	Trait class	<6	6–7	7.1–8	8.1–9	9.1–10	10.1–11	11.1–12	12.1–13	>13	5.7–14.0	ICMP 451	11.1
	No. of lines	6	12	23	24	18	11	11	8	3			

Qualitative and quasi-qualitative traits. Frequency distribution of the 114 restorer lines for 16 qualitative and quasi-qualitative characters revealed considerable variation for traits like anthocyanin pigmentation of first leaf, panicle exertion, panicle density, plant node pigmentation, panicle shape, seed shape and seed color. Majority of the lines (78%) were intermediate in their growth habit. Most of the restorer lines were non-bristled except for IPC 107, IPC 1617, IPC 804, IPC 1043 and IPC 408. Only 15 lines had pubescent node and 8 had pubescent leaf sheath. Green color was most dominant for node (56%) and internode (98%), brown color being the next major trait in node and red in the internode. Majority of the lines had gray colored seeds (76%) followed by cream (17%) and deep gray colored (6%) seeds. Obovate seed shape (56%) was the most dominant, followed by globular (40.5%) and only 1.7% lines had both elliptical and hexagonal seeds. About 75% of the lines had complete panicle exertion and 25% of the lines had variable exertion. Semi-compact panicles were most common (43%), followed by compact (21.5%) and loose panicles (15.5%); one line had very loose panicles and 16.3% lines showed variable expression within a line for very loose to very compact panicles. Only 6 lines had very compact panicles.

Within-line variability was observed in few restorer lines across two replications for some traits like anthocyanin pigmentation of seedlings, anther color, panicle exertion, panicle density, plant growth habit and plant node pigmentation. Eighteen R-lines had alternate phenotypes other than the predominant class for panicle density across both the seasons or in a single season. For instance, IPC 687 had compact panicles in rainy season but had loose panicles in dry season, whereas IPC 390 had loose to semi-compact panicles in dry season. Following the same trend, alternate phenotypes were present in 14 restorer lines for anthocyanin pigmentation of seedlings, either across both the seasons or in a single season. For instance, alternate phenotype for seedling pigmentation color was present in IPC 408 in both the seasons while IPC 976 had such alternate phenotypes in rainy season only. Similarly, for panicle exertion, alternate phenotypes were present in IPC 1000 in both the seasons. This within-line variability seems to be due to the method followed for their maintenance where panicles from 10 to 15 plants are bulked for these restorer lines. There is a possibility that the other restorer lines which have not shown such within-line variability in this study, might show it if tested with larger sample size, and perhaps in different environments. Therefore, there is every possibility that with larger number of plants tested for each of these restorer parents, one can come across a few plants that express the alternate phenotype of these qualitative (or quasi-qualitative) traits, which otherwise

should not be construed as a new phenotype or plant type at any given time. Existence of such variability within the line has a significant bearing on the protection of intellectual property.

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