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Advances in Pearl Millet Hybrid Breeding and Development of Parental Lines

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Abstract

Significant heterosis, stable male sterility systems, effective fertility restoration, and ease in selfing and crossing enabled breeders to enhance genetic gains in pearl millet through hybrid breeding. More than 50 years of breeding efforts in India and elsewhere utilizing germplasm from Asian and African regions have led to the development of a diverse array of trait- and adaptation-specific seed and restorer parental lines in productive backgrounds. Clarity on product profiles in breeding programs, maintaining significant genetic diversity among breeding lines, keeping seed and restorer parents in two separate heterotic pools, strong screening protocols for biotic and abiotic stresses, using molecular markers to select traits of interest, and utilization of tools to predict heterosis will be the key factors to move further in enhancing the hybrid potential of this crop in different regions of the world.

It is informed that consent has been taken for figures and graphs used in the current chapter.

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V. A. Tonapi et al. (eds.), *Pearl Millet in the 21st Century*,
https://doi.org/10.1007/978-981-99-5890-0_4

4.1 Introduction: Hybrids, a Better Option in Pearl Millet

In pearl millet, two types of commercial cultivars are available due to the presence of a high degree of outcrossing; it can be open-pollinated varieties (OPVs) or hybrids to exploit the heterosis to enhance the productivity in this crop. Pearl millet hybrids display a high degree of heterosis for grain yield, though OPVs permit only partial exploitation of heterosis. Currently, hybrids have occupied the majority of the area in Indian ecologies having an annual rainfall of >400 mm where the pearl millet crop is supported with adequate doses of fertilizers. Also, decade-long trial data from the Indian national cultivar release system have indicated that hybrids have shown yield superiority of about 25% over landraces/OPVs in highly drought-prone ecologies (Yadav et al. 2011).

Hybrid breeding provides an excellent opportunity to use diverse parental lines to harness maximum heterosis, and yet produce a cultivar with a high degree of morphological uniformity. The open-pollinating breeding system of pearl millet, high degree of heterosis, and availability of stable cytoplasmic-nuclear male sterility (CMS) and its fertility restorers have made the hybrid option commercially viable in pearl millet. The first commercial pearl millet grain hybrid HB-1, developed at the Punjab Agricultural University (PAU), Ludhiana, and released in India in 1965, had twice as much grain yield as the then-improved OPVs (Athwal 1965). Hybrids also offer a better option to manage downy mildew (DM), the most destructive disease in pearl millet. In single-cross hybrids, DM resistance generally lasts for 4–8 years. In India, due to strong hybrid programs (in the public and private sectors), there is significant cultivar diversity with about 70–80 hybrids on the farmers' field at any point in time. A high hybrid replacement rate coupled with resistance breeding has been able to contain DM disease. Currently, India is the largest grower of pearl millet hybrids (dual-purpose) in the world cultivating them on about 4.5–5.0 m ha, followed by the USA (mostly forage), and recently some West African countries have also introduced dual-purpose hybrids in their region. Hence, this chapter mostly draws strategies and examples of hybrid breeding in relation to Indian pearl millet programs owing to their long experience of hybrid breeding in this crop.

4.2 Development of Hybrid Parents

4.2.1 Trait Prioritization Under Different Product Profiles/Market-Segments

Pearl millet is cultivated under diverse agro-ecologies ranging from near-optimum environments (with high use of irrigation and chemical fertilizers) to extremely challenging drought-prone environments (with little external inputs). A single cultivar cannot be expected to perform well under all the environmental conditions, and a cultivar planted outside its adaptation zone would suffer yield reduction due to significant genotype × environment interactions. Therefore, breeding and evaluation require a subdivision of the testing environments into relatively more homogeneous groups of locations, called "mega-environments" or termed alternatively "market-segments," where specific genotypes can be targeted for individual mega-environment. For instance, the pearl millet cultivation area in India has been divided into three mega-environments (designated as A_1, A, and B zones) considering the geographical location, rainfall pattern, local adaptation, and other environmental conditions (Gupta et al. 2013). Clarity about the priority traits needed in different segments is a must, to enhance the efficiency of the breeding program and to develop a relevant product for any ecology.

Product profiles are an important tool for defining market segments and identifying the traits to be prioritized in a particular specific segment. Most of the pearl millet breeding programs in the public and private sectors in India have developed their product profiles (which may not be formally documented sometimes), depending on the need of their target mega-environment. For instance, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), as a Consultative Group on International Agricultural Research (CGIAR) center with a global mandate on pearl millet improvement programs, based in India targeting all the mega-environments to support Indian national breeding programs, has four product profiles, and they are depicted as an example in Table 4.1. Each product profile should have a list of "must-have" traits; these traits should essentially be present in the breeding lines bred under this product profile. There can be "long-term priority traits," which need to be addressed in 5–10 years. High grain yield, disease resistance, and maturity duration of 75–85 days, as per the agro-ecological requirements, have been accorded the highest priority in pearl millet (Yadav and Rai 2013). Because of the growing importance of dry stover for fodder purposes, there has been a considerable emphasis, in recent times, on breeding for dual-purpose cultivars producing both high stover and grain yields (Yadav et al. 2012b).

4.2.1.1 Seed Parents

In the breeding of seed parents (A-lines), the high grain-yield potential of A-lines, both as lines per se and in hybrids (i.e., combining ability), is the most important consideration. Thus, high yield/agronomic potential is the first target trait for which selection can be made visually in un-replicated nurseries. High yield, coupled with other agronomic and farmer-preferred traits, can be targeted in A-line breeding.

Some traits like lodging resistance, compact panicles, good exertion, and seed set can be considered for all environments, while region-preferred traits, like maturity, plant height, tillering ability, seed color, and seed size, are specific to different agro-ecologies. Most of these agronomic traits have high heritability for which visual selection during advanced generations is fairly effective. The d_2 dwarf plant height is the most dominant plant type concept in seed parent breeding. This has several operational advantages: (1) it provides the option for breeding hybrids of varying heights, (2) it provides greater control on seed yield and quality by reducing the risk of lodging that can occur under high management seed production conditions, and (3) it allows a much rapid detection and efficient rouging of off-types and pollen shedders in A-line. In addition, the A-lines must have complete and stable male sterility, and the B-lines must have profuse pollen production ability across the seasons and sites.

Table 4.1 Product profiles of ICRISAT's pearl millet breeding program (Asia), based at Hyderabad, India

Product profile	Estimated area (ha)	% area/ effort	Target agro-ecologies	Product development goals
(1) Parent lines of medium to late maturing, dual-purpose hybrids with disease resistance, for adaptation to better-endowed environments	4.0 m ha (rainy season)	65%	**Target**: India: East Rajasthan, central and South Gujarat, Haryana, Uttar Pradesh (UP), Maharashtra, peninsular India (400–700 mm/ annum)	**Must-have traits:** 1. Parents with high productivity and good general combining ability (GCA) for grain yield to develop hybrids with at least a 10% increase in grain yield (3–4 tons/ha) over representative checks (GHB 905, RHB 173, 86M01, 86M86, PAC 909) with improved fodder yield 2. 76–90 days maturity 3. Downy mildew (≤10%) and blast resistance (<3.0 score) 4. High grain iron (≥60 ppm) and zinc (≥35 ppm) content **Long-term priority:** 1. Lodging tolerance 2. Rancidity 3. N-use efficiency 4. Stover digestibility
(2) Parent lines of early maturing, dual-purpose hybrids with disease resistance, for adaptation to drought-prone environments	The total area is 3.5 m ha, *but this PC targets about 1.5 m ha through hybrids*	20%	**Target:** India: Western Rajasthan and drier parts of Gujarat and Haryana (200–400 mm/ annum)	**Must have traits:** 1. Parents with high productivity and good GCA for grain yield to develop hybrids with at least a 10% increase in grain yield (2.0–2.5 tons/ ha) over

(continued)

Table 4.1 (continued)

Product profile	Estimated area (ha)	% area/ effort	Target agro-ecologies	Product development goals
				representative checks (RHB-177, MPMH 17, HHB-67-improved) with improved fodder yield (~2.0 t ha^{-1}) 2. 65–75 days maturity 3. Downy mildew (≤10%) and blast resistance (<3.0 score) 4. Improved terminal drought tolerance 5. High grain iron (≥50 ppm) and zinc (≥35 ppm) content and better fodder quality **Long-term priority:** 1. Stover digestibility 2. Seedling-stage heat tolerance
(3) Parent lines of medium to late maturing, dual-purpose hybrids with disease resistance, for adaptation to high heat stress ecology in the summer season	0.6–0.8 m ha (and increasing)	5%	**Target**: India: North Gujarat, South Rajasthan, Western UP (summer season)	**Must-have traits:** 1. Parents with high productivity and good GCA for grain yield to develop hybrids with at least a 10% increase in grain yield (5–6 tons/ha) over representative checks (86M11, 9444) with improved fodder yield 2. 85–90 days maturity 3. Downy mildew (≤10%) and blast resistance (<3.0 score) 4. Flowering-

(continued)

Table 4.1 (continued)

Product profile	Estimated area (ha)	% area/ effort	Target agro-ecologies	Product development goals
				stage heat tolerance (summer season; >42 °C) 5. High grain iron (≥60 ppm) and zinc (≥35 ppm) content
(4) Cultivars and hybrid parents exclusively for forage and high biomass	1.0 m ha	10%	**Target**: India: Gujarat, Punjab, Rajasthan, UP, Madhya Pradesh, peninsular India (both summer and rainy seasons)	**Must-have traits:** 1. 10% increase in biomass yield over the best check (PAC-981) with comparable or improved fodder quality traits 2. Green biomass of 40–55 tons/ha and dry biomass of 15–20 tons/ha 3. Non-hairy leaves with a leaf/ stem ratio of 3:5 4. In vitro dry matter digestibility (IVDMD) of ≥50% with crude protein of ≥10% 5. Single cut (50–80 days); multi-cut (50–110 days) 6. Downy mildew (≤10%), rust (≤10%), and blast resistance (<3.0 score)

New plant types such as A-lines with long panicles (30–80 cm compared to standard normal of 10–20 cm), thick panicles (40–50 mm diameter compared to normal 20–30 mm), and large seed size (17–20 g of 1000-seed mass compared to standard normal of 9–12 g) are being developed with high productive ability in many pearl millet breeding programs in India. Progenies are evaluated for downy mildew and blast resistance during the generation advancement of breeding lines, which runs concurrent to agronomic evaluation to ensure that B- and R-lines finally produced are resistant to these diseases.

4.2.1.2 Restorer Parents

Restorer lines (R-lines) are used as pollinators to produce hybrids. These lines should (1) have good GCA, (2) restore complete male fertility in their grain hybrid, and (3) must produce profuse pollen that should remain viable at air temperatures as high as 42–44 °C. It is desirable to breed pollinators of 150–180 cm in height, but no shorter than the A-line with built-in attributes of panicle, maturity, and tillering that will be preferred by farmers in the hybrids. Besides being able to produce high-yielding hybrids, pollinators must have an acceptable level of lodging resistance and should also possess adequate levels of resistance to various diseases.

4.2.2 Genetic Diversification

A range of germplasms from India and Africa with diverse phenotypic characteristics, such as tillering, panicle size, earliness, grain size, grain color, and plant height, was strategically exploited to diversify the genetic base of both seed and restorer parents (Andrews and Kumar 1996; Rai et al. 2009a; Yadav et al. 2012c; Patil et al. 2020). In the last five decades, hybrid breeding in pearl millet has received a very high priority in India using genetically diverse parental lines targeting various production ecologies that have helped to intensify the genetic gains (Rai et al. 2009a; Yadav et al. 2012a, 2021).

Seed Parents Considering the agro-ecological conditions and farmers' preference for specific segments, a trait-based approach in breeding seed parents with high grain yield potential and high levels of DM resistance has been followed, resulting in the development of large diversity in seed parents. For instance, the utilization of diverse breeding materials in the development of 104 designated B-lines at ICRISAT from 2005 to 2018 has resulted in a wide range of variability. For instance, days to 50% flowering ranged from 41 to 64 days, plant height from 72 to 156 cm, number of productive tillers plant^{-1} from 1 to 9, panicle length from 14 to 37 cm, panicle diameter from 1.7 to 3.8 cm, and 1000-grain weight from 5.4 to 16.0 g in these 104 B-lines (Gupta et al. 2022a). Such a large morphological diversity originated from the focused selection for these traits. To enhance the genetic diversity, it is very important to continuously utilize diverse germplasms in the breeding pipeline; for instance, among the 203 A/B pairs developed at ICRISAT from 1981 to 2018, 17 B-lines were directly selected from the composites, 25 lines were derived from crosses that involved germplasms in their parentage, and 37 lines had composites in their parentage. Thus, 79 seed parental lines had some components of germplasm and/or composite in their parentage, indicating apparently substantial usage of germplasms/composites in the development of these seed parental lines. The remaining 124 seed parental lines were derived from crosses between elite inbred lines (Table 4.2).

New germplasms, like landraces and germplasms from gene banks or improved OPVs, can be strategically involved in the B-line breeding program based on the traits required in seed parents and also considering cytoplasmic maintenance

Table 4.2 Type of material used in the development of designated maintainer (B) lines (1981–2018) and restorer (R) lines (2006–2019) of pearl millet bred at ICRISAT-India

Type of genetic material	Number of lines derived		Remarks
	B-lines (203)	R-lines (122)	
Germplasm	3	2	Inbreeding and selection directly from germplasm
Composites	17	66	Includes composites and open-pollinated varieties
Germplasm × Elite line	22	6	Includes early-generation breeding lines derived from crosses between germplasm and elite lines
Composite × Elite line	37	6	Includes early-generation breeding lines derived from crosses of composites and elite lines
Elite line × Elite line	124	42	Includes cross between elite lines from advanced generations

reaction. Traits of importance, like desirable maturity period, panicle and grain traits contributing toward high yield, and photo-insensitiveness, help to identify germplasms for utilization in seed parent breeding programs. For example, germplasm from the Togo region of West Africa, known as "*iniari*" germplasm, having early maturity, considerable photo-insensitiveness (required for successful seed production and for stable hybrid performance in different agro-ecologies), bold grains, and dark gray color, was identified for its utilization in B-line breeding, and hence most of the programs developing B-lines have significantly used these genetic backgrounds (Andrews and Kumar 1996; Singh and Gupta 2019). At the beginning of a hybrid breeding era in pearl millet (the 1960s), trait-based composites and populations were observed for traits and fertility/sterility and were involved in seed parent or restorer parent development programs.

Restorer Parents Like seed parents, it is essential to enhance genetic diversity in the restorer lines. Breeding programs in the public and private sectors have introgressed significant genetic variability for different traits in the restorer lines. For instance, 122 R-lines bred at ICRISAT during 2006–2019 had plant height ranging from 100 to 179 cm and days to 50% flowering varied from 45 to 60 days, wherein 43% of lines were of medium duration, 35% were late, and 13% were very late, while only 9% of the lines were of early flowering. Panicle length varied from 12.7 to 31.1 cm wherein 53% of lines were small and 46% were medium. Panicle diameter varied from 1.4 to 3.8 cm wherein 74% were medium, 24% of lines were thick, and only 2% of lines showed thin panicles. Thousand-grain weights varied from 6.0 to 11.9 g wherein 72% of lines were medium, 20% were bold, and only 8% showed small grain size (Gupta et al. 2022b). Diverse germplasms should also be continuously utilized to enhance genetic diversity in the development of R-lines; for example, the parentage of mentioned 122 designated restorer lines at ICRISAT showed the utilization of a wide range of germplasms and improved lines in developing these R-lines. For instance, 67 R-lines were selected directly from trait or adoption-specific composites, and two R-lines were directly selected from the germplasm accessions (Table 4.2). In addition, six R-lines were derived from crosses that involved germplasms in their parentage, and seven lines had composites in their parentage. Thus, 82 restorer lines had some components of germplasm and/or composite in their parentage, indicating apparently substantial usage of germplasms and composites in the development of these R-lines. The remaining 44 R-lines were derived from crosses between elite inbred lines.

4.2.3 Cytoplasmic Diversification

4.2.3.1 CMS Search and Characterization

The entire hybrid development program has been (and still continues to be) based largely on the single CMS system (A1 system) in India and around the globe. This large-scale cytoplasmic uniformity exposes the hybrid pearl millet seed industry to the potential disease and insect-pest epidemics. Thus, efforts were made to assemble CMS sources developed elsewhere, initiate a search for new CMS sources, and characterize these for the stability of male sterility, their maintainer frequency in the germplasm, character association, and any possible impact on restorer line development. Initial evaluation of A-lines for pollen shedders (a measure of the stability of male sterility) had shown that the A2 and A3 sources were most unstable. Further evaluation of A-lines with other CMS sources during the rainy, and mild and hot summer environments showed that A-lines with Aegp cytoplasm (0.0–0.1% pollen shedders, depending on the nuclear genetic backgrounds and the environments in which A-lines were evaluated), and those with the A4 cytoplasm (0.0–0.3% pollen shedders) were more stable than those with the A1 cytoplasm (0.0–2.5% pollen shedders) (Rai et al. 2001). The A-lines with the A5 cytoplasm had no pollen shedders, implying that the A5 CMS system was the most stable. An additional advantage is that the genetic background of male-sterile lines in A_4 and A_5 cytoplasm does not affect the fertility restoration of hybrids, whereas the genetic background of A_1 cytoplasm has a significant effect on the fertility restoration (Rai et al. 2001, 2009b; Gupta et al. 2010). If restorer development for A4 and A5 CMS has to be enhanced, it would require a major shift in breeding efforts as most of the restorers in all the hybrid programs in India and elsewhere are of A1 CMS. But there is a serious lack of A_4 restorers and A_5 restorers in elite agronomic backgrounds (Gupta et al. 2012, 2018). However, excellent genetic stocks of A_4 and A_5 restorers have been developed at ICRISAT, which are used in backcross breeding to develop restorer lines of A_4 and A_5 CMS systems (Rai and Rao 2003; Rai et al. 2006).

New germplasms must be continuously involved in the breeding pipeline to enhance the genetic diversity of restorers. In one such effort, topcross hybrids developed between A-lines with the A1, A4, and A5 cytoplasm and several improved populations from Asia and Africa regions showed that the frequency of maintainers was highest for the A5 system (mean of 86%), followed by A4 system (37%), and it was the lowest for the A1 CMS system (7%), thus providing the prospects of greatest genetic diversification of A-lines with the A5 system, and then with the A4 and the A1 systems, in that order (Fig. 4.1) (Patil et al. 2020).

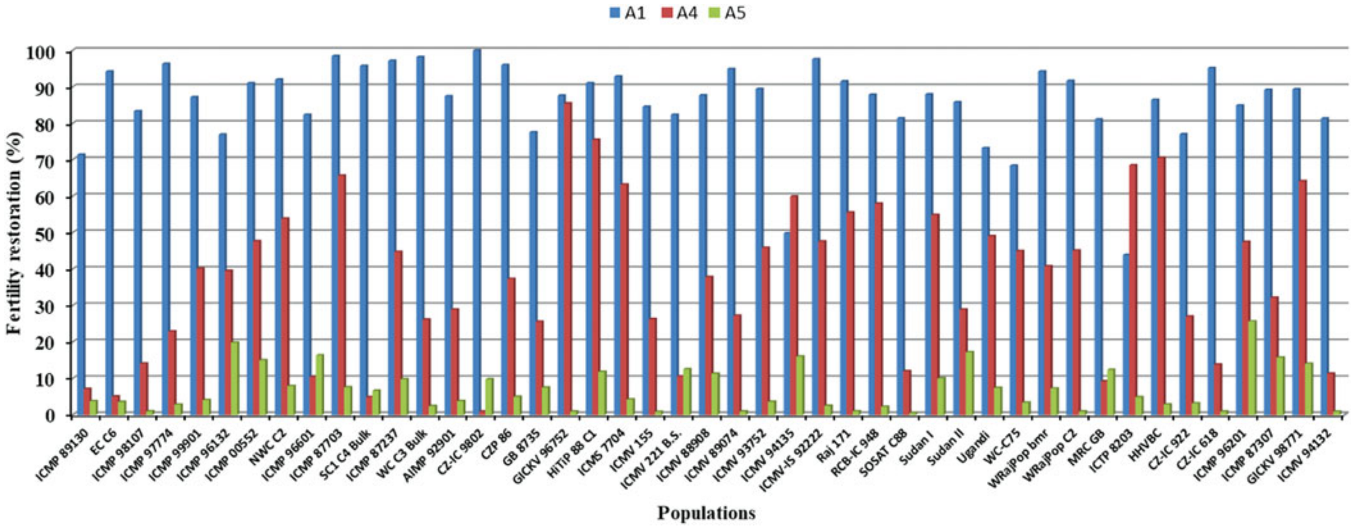


Fig. 4.1 Fertility restoration percentage in topcross hybrids of A_1, A_4, and A_5 made with 45 diverse African and Asian bred/origin pearl millet populations

4.2.3.2 Cytoplasmic Diversification of Seed and Restorer Parents

The public and private sector breeding programs continue using the A1 CMS system, due to the lesser availability of restorer parental lines of the A4 and A5 cytoplasm. There has been an increasing emphasis in different breeding programs on the utilization of the A4 and A5 CMS systems in seed parent's development, with a parallel effort on developing their restorers. For instance, of the 203 designated A-lines developed from 1981 to 2018 at ICRISAT-India, 108 are based on the A1 cytoplasm, 81 on the A4 cytoplasm, and 14 on the A5 cytoplasm. This provides a wide range of genetically diverse A-lines of these two new and most stable CMS systems. A/B pairs can be developed and designated on more than one CMS system, if possible. Out of the 104 A/B pairs developed from 2005 to 2018 at ICRISAT, 32, 41, and 10 were designated only on A_1, A_4, and A_5 CMS systems, respectively; while 7, 2, and 4 A/B pairs were developed simultaneously on 2 CMS systems: A_1 and A_4, A_1 and A_5, and A_4 and A_5, respectively. Four A/B pairs were developed simultaneously on three A_1, A_4, and A_5 CMS systems.

Considering the large availability of restorer lines of A1 CMS across the breeding programs in the public and private sectors and simultaneously to diversify the restorer base, the focus is on the order of A1 followed by A4 and A5 restoration. For instance, out of the 122 restorer parents bred at ICRISAT from 2006 to 2019, 102, 60, and 11 R-lines were restorers of A_1, A_4, and A_5 CMS (Cytoplasmic Male Sterility) systems, respectively (Gupta et al. 2022a, b). Some lines are restorers for more than one CMS system, like 41 out of mentioned 122 lines were dual restorers for A_1 and A_4, and 2 R-lines for both A_1 and A_5 CMS systems. Four R-lines were restorers of all three A_1, A_4, and A_5 systems. Following the same strategy, public and private sector breeding programs in India have diversified their cytoplasmic base and developed a large number of seed and restorer parental lines on different CMS systems.

4.2.4 Breeding Methods

Hybrid parent breeding program targets to develop productive B- and R-lines with combinations of numerous agronomic and adaptation traits. Once this is achieved in B-lines, conversion of B-lines into A-lines is followed through backcross conversion.

4.2.4.1 Line Breeding to Develop B- and R-Lines

For both B- and R-line breeding, the first planting of F2 populations and composites/ OPVs generally can have 500–1000 plants depending upon the diversity of parental lines involved in the cross and on the extent of variation in the population. For crosses or populations found outstanding, bigger populations can be planted in subsequent seasons to make more rigorous selections. Pedigree breeding should be followed generally up to F7/S5 stage after which bulk breeding might be carried out in lines that have attained high levels of uniformity for morphological traits and disease (DM and blast) resistance. At least one DM/blast screening of breeding lines should have been completed by the time the F7/S5 breeding stage is reached. The screening at this stage is done against the pathogen-isolate representing the ecology for which the cross is targeted to develop breeding lines. At each inbreeding stage, about 30–50% of breeding lines not found acceptable on the multiple-trait criteria can be discarded. However, lines found outstanding for even one trait are selected for further use in the crossing program. F5/S3 onward, selected lines can be evaluated in the plantings according to parentage, but highly selected lines from various populations are also classified into trait-specific groups. Inbred lines derived from different crosses can then be crossed to initiate a new cycle of inbreeding and selection for inbred line development.

While developing inbred lines for use as hybrid parents, there are three important considerations: (1) selection for performance per se with respect to yield potential, disease/insect pest resistance, and adaptation, (2) selection for fertility restoration ability in R-line breeding stream and sterility maintenance in B-line breeding stream, and (3) evaluation for combining ability. Selection for performance per se for priority traits (yielding ability, apparent grain quality traits, downy mildew, and blast resistance) in the breeding nurseries can be made at all inbreeding stages. In addition, selection for downy mildew or blast resistance using the greenhouse inoculation technique can be made at least at two inbreeding stages from F_5 onward. Test for fertility restoration/sterility maintenance reaction should be conducted at F_5/F_6 generation when the lines become reasonably uniform.

4.2.4.2 Selection for DM and Blast Resistance

Periodic evaluation of progenies for DM and blast resistance during the course of inbreeding and selection should run concurrent to agronomic evaluation to ensure that lines (B-lines and restorer lines [R-lines]) produced finally are resistant to this disease. Diverse pathotypes should be maintained in the greenhouse screening facilities. Results have shown that an un-replicated single-pot screening of breeding lines (approximately 30–40 seedlings per line) is highly effective for rejecting the susceptible lines, as the correlation between the single-pot screen and two-pot screen for DM incidence is highly significant ($r = 0.75^{**}$) (Rai et al. 2004).

Trait-specific breeding lines should be evaluated for DM and blast resistance against pathotypes from the region for which the lines are targeted. However, breeding lines in some trait-specific groups (e.g., early maturity, medium seed size, and average tillering with long panicles) should be evaluated in successive steps against more than one diverse pathotype because of the wider requirements of such materials.

The same trait-based selection approaches combined with high yield potential, DM, and blast resistance have also been followed for restorer line breeding as for the A-line breeding. One major difference is that in restorer breeding, there is a negligible effort on breeding dwarf R-lines. For DM and blast resistance, the same procedure is applied as in seed parent breeding.

4.2.4.3 Testing for Combining Ability

Early-generation testing has been recommended to select progenies for high general combining ability. B- and R-breeding lines at $F_{4/5}$ or $S_{2/3}$ stage are crossed to the tester(s) (from the opposite side of seed and restorer pool and identified for specific target ecology) in line × tester design, and test crosses should be evaluated in target ecology. Based on the multilocation evaluation of test crosses, lines with good GCA are identified. B-lines should be tested for GCA before they are used to develop A-lines to prevent unnecessary backcrossing efforts. The identified good combining B-lines are essentially converted to A-lines, while B-lines having high performance per se are also converted to A-lines. Selection is practiced for performance per se during inbreeding generations, and it has been observed in pearl millet that the correlation between performance per se of the lines and their general combining ability for grain yield is either non-significant or positive and significant (Rai and Virk 1999; Singh and Gupta 2019; Gupta et al. 2020). This would imply that high general combiners are more likely to be found in a high-yielding group of lines than in the low or average-yielding groups. Further, the high-yielding ability of parental lines, especially A-lines, is very important from the viewpoint of the seed production economy.

4.2.4.4 A-Line Development

Inheritance of male sterility maintenance, whether simple or complex, affects the efficiency and cost-effectiveness of the conversion process: the simpler the inheritance, the greater the selection response. A_1 CMS system is reported to be under single-gene control of male sterility maintenance and fertility restoration, but simultaneously the possibility of two- and three-gene models governing this trait is also not ruled out (Yadav et al. 2010). Also, dominant single-gene control of fertility restoration has been revealed for A_4 fertility restoration (Gupta et al. 2012), and trigenic inheritance of male fertility restoration was suggested for A5 CMS, where dominant alleles at any two of the three duplicate complimentary loci will lead to fertility restoration (Gupta et al. 2018).

Conversion Stage

Conversion of potential B-lines into A-lines can be initiated once it has been ascertained that they meet the multiple criteria of yield potential, agronomic traits, DM resistance, and high levels of morphological uniformity. Normally, at least three generations of progeny-based evaluations (i.e., at least up to F5/S3) must precede before undertaking them for conversion into A-lines, which translates to about 2 years × two seasons of prior field evaluation on a plot basis. In exceptional cases (either exceptional performance or any urgency to save critical time), a progeny with outstanding performance can be put into an A-line conversion scheme at an earlier inbreeding stage.

Conversion Method

Since highly uniform B-lines are used for conversion, crosses and backcrosses are made on a line basis rather than on an individual plant basis. Following this method, it has been observed that there is neither significant variability within B-lines for agronomic and adaptation traits nor any variability within the F1 and backcross progenies for male sterility, thus eliminating any need for within-plot selection. This method of conversion of B-lines into A-lines also reduces unnecessary work needed while dealing with sister A/B pairs in the plant × plant backcross conversion scheme and allows for the maintenance of greater diversity for the equivalent resources used in the plant × plant crossing scheme. Even with the use of advanced generation B-lines for conversion, lines found to have produced fully sterile F1s sometimes produce backcross progenies with low frequency of pollen shedders. First, such cases are very rare. Second, unless the B-line is truly outstanding, plant × plant crossing in backcross progenies is not done, and those backcross/B-line pairs are eliminated from the crossing program. Four to six backcrosses may be adequate if the new B-line and the cytoplasm donor CMS line are closely related, while up to eight backcrosses may be needed for more diverse lines.

Selection During the Conversion Process

During the conversion process, selection continues, but more among the B-lines than within the B-lines, especially during the advanced backcross stages. Generally, there can be about 20% rejection of the B-lines and their corresponding F_1 and backcross progenies at each backcross stage. Each plant in the F_1 and backcross progenies is also selfed (using the tiller panicle) to score for and confirm complete sterility. One or two generations of backcross time can be saved by selecting plants in the backcross progenies that have a greater resemblance to the basic plant morphology of the recurrent parent (B-line). This, however, would require planting larger plots of the backcross progeny (say 80–100 plants rather than the customary 20–25 plants) to make the selection more effective.

4.2.4.5 Improving Adaptation and Nutritional Traits

Drought and high heat stress challenge pearl millet in various production environments. While drought affects the crop across agro-ecologies, heat stress assumes importance in specific regions. Drought resulting from low rainfall or due to its erratic distribution is the primary abiotic production constraint. Drought affects the grain-filling stage the most, as both grain number and grain size are significantly reduced when the crop is exposed to drought stress at this stage (Fussell et al. 1991). Dissection of drought tolerance in terms of physiology, phenology, and morphology of the crop has led to the understanding of the yield formation process under drought (Van Oosterom et al. 2003; Yadav 2011), helping breeders to identify and target specific traits in different drought environments. It is very challenging to use physiological traits as selection criteria for drought tolerance, especially when dealing with many genotypes in breeding nurseries. Drought escape has been the most exploited mechanism by targeting early maturity for getting greater genetic gains in drought-prone regions (Yadav et al. 2011). Morphological traits such as high tillering, small grain size, shorter grain-filling periods, and high panicle harvest index are the most desirable and scorable traits that can be measured easily and have been manipulated successfully in breeding programs as there is an abundant variation available for these traits (Yadav et al. 2017).

Adapted landraces with elite genetic backgrounds should be involved in the hybridization to create new gene combinations that can lead to amalgamating of adaptation to stress environments and high productivity (Presterl and Weltzien 2003; Yadav and Rai 2011; Patil et al. 2020). Genome regions underlying drought tolerance-related traits have been identified and mapped (Yadav et al. 2002, 2004; Serba and Yadav 2016), and several such genomic regions are being manipulated to enhance drought tolerance (Bidinger et al. 2007; Sharma et al. 2014).

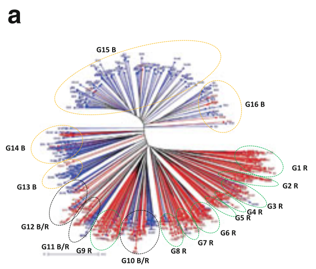
During the last decade, pearl millet has emerged as a highly productive and remunerative crop in the hot and dry summer season in the northern and western parts of India. With higher air temperatures (often >42 °C) coinciding with flowering in this season, the crop suffers from reproductive sterility, leading to drastic reductions in seed set and finally lesser grain yield (Gupta et al. 2015; Djanaguiraman et al. 2018). Heat tolerance at the reproductive stage has emerged as an important target trait to enhance genetic gains. Flowering-period heat stress screening protocols have been standardized for screening under controlled environment facilities (growth chambers) and field conditions in heat stress-prone target ecology (Gupta et al. 2015). Multilocational and multiyear field screening in the summer season involving a large number of hybrid parental lines, germplasm accessions, and improved populations established that: stigma is more heat-sensitive than pollen; large genetic variation exists between breeding lines and within open-pollinating populations; the boot-leaf stage is more heat-sensitive than a panicle-emergence stage; and heat tolerance behaves as a dominant trait. These screenings led to the identification of heat-tolerant breeding materials and can further be used to enhance heat tolerance (Gupta et al. 2016, 2019) and facilitate the pyramiding of heat tolerance in high-yielding hybrids.

Pearl millet has been considered a highly nutritious cereal with higher levels of proteins and several minerals than other major cereals; hence the goal of core breeding has been focused on increasing the yield potential. About 24.3% protein content has been reported in the germplasm and up to 19.8% in elite breeding lines (Singh et al. 1987). However, no serious efforts were made to improve protein content because of its negative correlations with grain yield (Singh and Nainawatee 1999). Recently, given the global recognition of widespread deficiencies of iron (Fe) and zinc (Zn), these nutritional traits have been added to the breeding objectives. A large variability has been indicated for Fe and Zn content in germplasms and breeding lines, suggesting the feasibility of genetic enhancement for these micronutrients in pearl millet. Screening of more than 120 Indian commercial hybrids has shown 46–56 ppm Fe and 37–44 ppm Zn (Rai et al. 2016). The daily recommended allowances for Indian adults are 17–21 and 10–12 mg/day for Fe and Zn, respectively. Fe and Zn contents in pearl millet are largely governed by additive genetic variance (Govindaraj et al. 2013; Kanatti et al. 2014), suggesting that both parental lines of hybrids would be required to improve for these micronutrients. Relatively lower G × E influences on the accumulation of Fe and Zn in pearl millet grains (Kanatti et al. 2014; Govindaraj et al. 2016) also indicated the effectiveness of progeny selection in the pedigree breeding to develop lines with increased levels of grain Fe and Zn densities. These two micronutrients are highly positively correlated to each other, and also have a positive and highly significant correlation with seed size (Gupta et al. 2009; Kanatti et al. 2014; Govindaraj et al. 2016). These associations would give breeders an opportunity to develop Fe- and Zn-rich cultivars with large grain size irrespective of their color and to allow enhancement of micronutrients in mainstream breeding.

4.3 Enhancing Magnitude of Heterosis

4.3.1 Heterotic Groups

A narrow genetic base is one of the major constraints to enhance the genetic gains for most of the hybrid breeding programs. To address this, a systematic assessment of genetic diversity (by phenotyping and/or genotyping) to develop heterotic groups is needed to develop hybrids with higher standard heterosis. The fundamental principle for the exploitation of heterosis is to characterize the germplasm into different heterotic groups, which helps breeders to develop inbred lines and use the available germplasm more efficiently for maximizing hybrid breeding outcomes (Melchinger and Gumber 1998). In pearl millet, a diverse range of breeding material has historically been used to develop either seed parents (B-lines) or restorer parents (R-lines), depending upon their specific phenotypic traits (Rai et al. 2006). Studies assessing molecular diversity classified such lines into genetically distinct groups and confirmed the existence of two broad-based pools in hybrid parents—one for seed parents and another for restorer parents (Nepolean et al. 2012; Gupta et al. 2015; Singh et al. 2018). The existence of B- and R-lines as separate groups has been found responsible for behaving as two separate broad heterotic pools, as B × R hybrids reported significantly higher levels of heterosis than B × B or R × R hybrids (Singh et al. 2018). Another study involving 320 R- and 260 B-lines derived from six major pearl millet breeding programs in India identified heterotic groups based on the heterotic performance and combining ability (Gupta et al. 2020) (Fig. 4.2). Hybrids



b

Sl.No.	Cross combination of marker groups	Genetic distance	Hybrid Yield (kg ha^{-1})	MPH (%)	BPH (%)	Yield advantage over ProAgro 9444 (%)	Yield advantage over Pioneer 86M86 (%)
1	G12B x G12R	0.25	4507.4	152.5	70.9	29.2	10.6
2	G10B x G12R	0.31	4456.1	96.0	64.1	27.7	9.3
3	G13B x G12R	0.31	4434.3	116.7	68.1	27.1	8.8

Fig. 4.2 (**a**) Clustering pattern based on restricted site-associated DNA (RAD) sequencing of 540 B- and R-lines; (**b**) identified heterotic groups. (Source: Gupta et al. 2020)

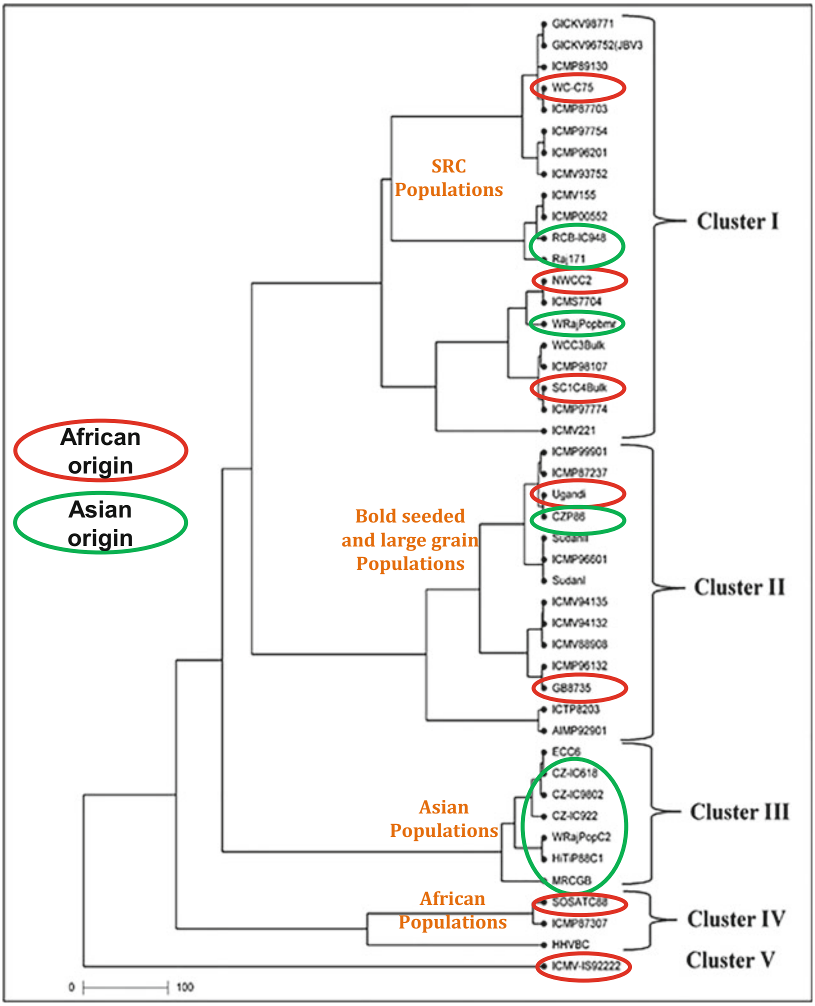


Fig. 4.3 Population heterotic pools formed among 45 African and Asian origin/bred pearl millet improved populations

from these identified B × R heterotic groups showed grain yield heterosis of more than 10% over the best available commercial hybrid checks. This study also indicated that distinct parental groups could be formed based on molecular markers, which can help in assigning the hybrid parental lines into heterotic groups to develop high-yielding hybrids. Now, appropriate testers to categorize the new hybrid parental lines or new germplasm into these heterotic groups have been identified to further enhance the heterosis (Unpublished PhD. Thesis 2022, P. Rakshith, ICRISAT). Moving beyond the existing variability in available inbreds, as shown in Fig. 4.3, heterotic pools have been formed among African and Asian origin/bred pearl millet improved populations to help breeders enhance genetic diversity in the future hybrid parental lines (Patil et al. 2020). New populations can be identified for developing heterotic parental lines using these results.

Most public and private sector pearl millet breeding programs have been advancing progenies based on performance per se of lines to develop high-yielding seed parental lines. Hence, there is not much information available about the extent of specific combining ability (SCA) and GCA variances in the existing B- and R-gene pools of pearl millet. A study has shown a high SCA:GCA ratio (about two times), indicating the predominance of SCA variance over GCA variance in pearl millet hybrid parents, which is quite different from many of the established maize hybrid breeding programs of the USA and Europe, where low SCA:GCA ratio was observed (Gupta et al. 2020). Higher values of GCA or high ratio of GCA:SCA enable breeders to predict the breeding value of their lines and hybrids with higher accuracy. Considering this scenario in other crops, there is a need to investigate SCA and GCA variances in the existing B- and R-line heterotic pools of pearl millet to better understand the contribution of GCA and SCA variances towards heterosis.

4.3.2 Predicting Heterosis

Identifying promising hybrids requires generating and testing large numbers of hybrids under different field conditions. At first, performing crosses to generate F1 hybrids is a labor-intensive process, and further evaluating hybrids across field trials under several environments requires significant time and resources. Hence, methods for selecting parental inbred lines and determining which crosses are likely to yield the best hybrids are a critical part of crop improvement. Traditionally, mid-parental values have been a common way to predict the performance of hybrids based on inbred values, combined with estimates of general combining ability (GCA) (Gowda et al. 2013; Xing et al. 2014; Mühleisen et al. 2015). However, for traits where significant heterosis exists, the phenotypes of hybrids can vary significantly from what would be predicted using mid-parent values and estimated GCA. In these cases, it can be necessary to estimate specific combining ability (SCA) values for each potential cross. The incorporation of genetic markers can improve the accuracy with which both GCA and SCA can be predicted by enabling the sharing of data across multiple tested lines carrying common haplotypes (Schrag et al. 2007). When applied to sets of genetic markers across the whole genome, this process is referred to as genomic prediction (GP), which can be used to implement breeding programs based on estimated breeding values from genome-wide sets of markers, a process known as genomic selection (GS). In a study on pearl millet, Liang et al. (2018), based on the data on inbreds and hybrids, reported prediction accuracy ranging from 0.73 to 0.74 for 1000-grain weight, 0.87 to 0.89 for days to flowering time, 0.48 to 0.51 for grain yield, and 0.72 to 0.73 for plant height. This study also indicated that even small numbers of selected single nucleotide polymorphisms (SNPs) can achieve relatively high prediction accuracy in the pearl millet population. The implementation of a hybrid GS/GP-guided pearl millet breeding program has the potential to improve the efficiency of breeding efforts significantly.

4.3.3 Molecular Breeding

Genomics and genomics-assisted breeding can help improve the precision and efficiency of the hybrid breeding program. Several efforts like sequencing of Pearl Millet Inbred Germplasm Association Panel, mapping population parents, and elite hybrid parental lines have led to the development of a huge (>32 million) repository of genome-wide SNPs, which can help in trait discovery, mapping, and deployment of quantitative trait loci (QTLs)/alleles/candidate genes linked to traits of economic interests. It also has helped with the development and implementation of prediction models for the pearl millet (Jarquin et al. 2020; Liang et al. 2018). Now, the need is to map and deploy genes of agronomic importance and re-sequence lines to mine and map genes of interest, and superior haplotypes for different traits can be identified to form the basis of haplotype-based breeding (Sinha et al. 2020). Many traits of agronomic importance have been mapped related to diseases, terminal drought, grain and fodder quality, combining ability loci, and heterotic gene pools (Kumar et al. 2018; Basava et al. 2019; Gupta et al. 2020; Srivastava et al. 2020). With simple sequence repeats (SSR) and SNP markers in pearl millet, these QTLs can be remapped using the currently available SNP-based high-throughput genotyping systems. This will allow integration into the modern breeding pipelines using high-throughput genotyping platforms available currently in pearl millet. Furthermore, it has become possible now to map breeding-related traits quickly with the availability of reference genome sequence, large-scale whole genome resequencing data, cost-effective genotyping platforms, and precise phenotyping platforms (Bohra et al. 2020). We believe the near future will witness the deployment of genomic breeding approaches such as haplotype-based breeding, forward breeding, genomic prediction, and gene editing for pearl millet improvement (Varshney et al. 2020).

4.4 Hybrid Breeding for Different Regions

Apart from India, many countries are now working toward the development of pearl millet hybrids, and hybrids have been released in a few countries. Grain and stover yield, and DM resistance are high-priority traits in both African regions (Eastern and Southern Africa [ESA], and West and Central Africa [WCA]). The parasitic weed *Striga hermonthica*, stem borer (*Coniesta ignefusalis*), and head miner (*Heliocheilus albipunctella*) are the additional biotic constraints in the WCA region, and thus need to be addressed.

Hybrids bred at ICRISAT-India have shown high yields and adaptation in the ESA (Eastern and Southern Africa) region; hence, India bred materials could be of direct and indirect use for hybrid breeding programs in the ESA region, and vice-versa to some extent. This is not true in the WCA region, so the breeding materials and parental lines developed in India will be of no direct use in the WCA region. African pearl millet programs will have to develop in-house potentially useful breeding lines and potential hybrid parents and test for their utility in the target regions. The initial thrust in African regions should be placed on addressing relatively better-endowed environments with fewer high-priority traits, considering all the factors that increase the probability of success in the medium term. Since hybrid cultivar programs of large size are not likely to be in place in the African regions soon, the frequent cultivar replacement strategy for DM management is not a likely option. The strategy for this region should be to strengthen the DM resistance breeding program (supported by marker-assisted selection [MAS]) and implement alternative hybrid options.

In the case of forage hybrids, which are quite popular in the USA, Brazil, and some central Asian countries, the use of A5 CMS should help to develop a range of seed parents due to its high maintenance potential in any genetic background, and also that crop homogeneity and fertility restoration is not required in forage hybrids.

4.5 Conclusions

Continuous enhancement of genetic diversity in hybrid parental lines has been the key driver of success behind pearl millet hybrid breeding in India. Strategic involvement of diverse germplasms to develop seed and restorer parental lines can help maintain sufficient genetic distance between seed and restorer parental lines to enhance heterosis. Cytoplasmic diversification is critical to broaden the genetic base of germplasms involved in hybrid breeding, while simultaneously providing protection to hybrids from the risk of cytoplasmic breakdown to the future virulent variants of pests and diseases. The breeding pipeline needs to be integrated with robust screening protocols for biotic and abiotic stresses to develop climatic resilient hybrids. Nutritional traits should be mainstreamed into the breeding pipeline to develop not only productive but also nutritionally rich hybrids. New tools should be tested rigorously and adopted in breeding programs to introgress new desirable alleles and also to predict heterosis to support the new-generation breeders. New hybrids would offer a robust solution to climate change by expanding pearl millet in new regions globally. To achieve this, the genetic gains in pearl millet can be further enhanced with the judicious use of conventional and modern tools.

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