Received: 5 August 2019 | Revised: 28 May 2020 | Accepted: 3 June 2020

DOI: 10.1002/csc2.20245

Crop Science

ORIGINAL RESEARCH ARTICLES

Crop Breeding & Genetics

African and Asian origin pearl millet populations: Genetic diversity pattern and its association with yield heterosis

K. Sudarshan Patil[1,2] | **Shashi Kumar Gupta**[1] | **Balram Marathi**[2] | **Shashibhushan Danam**[2] | **Ramesh Thatikunta**[2] | **Abhishek Rathore**[1] | **Roma Rani Das**[1] | **Kuldeep Singh Dangi**[2] | **Om Parkash Yadav**[3]

[1] International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324, Hyderabad, Telangana, India

[2] Professor Jayashankar Telangana State Agricultural University, Hyderabad 500 030, Rajendranagar, Telangana, India

[3] ICAR-Central Arid Zone Research Institute (CAZRI), Jodhpur, Rajasthan, India

Correspondence
K. Sudarshan Patil, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT), Patancheru 502 324 Hyderabad, Telangana, India.
Email: sudarshan.gpb@gmail.com

Assigned to Associate Editor Leah Mchale.

Abstract

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is a staple food crop of arid and semi-arid regions of Asia and Africa. Forty-five pearl millet populations of Asian and African origin were assessed for genetic diversity using 29 simple sequence repeat (SSR) markers. The SSR-based clustering and structure analyses showed that Asian origin–Asian bred (As-As) and African origin–African bred (Af-Af) populations were distributed across seven clusters, indicating no strong relationship among populations with their geographical origin. Most of the African origin–Asian bred (Af-As) populations had a higher average number of alleles per locus than As-As or Af-Af populations, and the majority of them clustered separately from As-As or Af-Af populations, indicating that introgression of African origin breeding materials led to the development of new gene pools adapted to the Asian region. Fourteen populations representing seven clusters were crossed according to a diallel mating design to generate 91 population hybrids (seeds of direct and reciprocal crosses were mixed) and evaluated at three locations in 2016. All the 91 hybrids when partitioned into three groups based on genetic distance (GD) between parental combinations (low, moderate, and high), revealed no correlation between GD and panmictic midparent heterosis in any of the groups, indicating that grain yield heterosis cannot be predicted based on GD. Two population hybrids (GB 8735 × ICMP 87307 and Sudan I × Ugandi) exhibited high levels of yield heterosis over standard checks and can be further utilized using different breeding schemes to develop high-yielding pearl millet cultivars.

Abbreviations: (As × Af)-As, (Asian x African) origin–Asian bred; Af-Af, African origin–African bred; Af-As, African origin–Asian bred; AMOVA, analysis of molecular variance; As-As, Asian origin–Asian bred; GD, genetic distance; HWE, Hardy–Weinberg equilibrium; ICMP, ICRISAT millet pollinator; ICMV, ICRISAT millet variety; ICRISAT, International Crops Research Institute for the Semi-Arid Tropics; MCMC, Markov chain Monte Carlo; MRC, Mandore restorer composite; MRD, modified Roger's distance; OPV, open-pollinated variety; PCH, panmictic commercial heterosis; PCR, polymerase chain reaction; PMPH, panmictic midparent heterosis; QTL, quantitative trait locus; SRC, smut-resistant composite; SSR, simple sequence repeats.

1 | INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.], with a global area of ~34 million ha and production of ~31 Tg, ranks sixth among cereals after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), and sorghum [*Sorghum bicolor* (L.) Moench] (FAO, 2020). The majority of the pearl millet area is in Africa (~22 million ha) and Asia (~11 million ha). Recently, the crop has expanded to nontraditional areas, such as Brazil (De Assis, De Freitas, & Mason, 2018), the United States, Canada, Mexico, West Asia and North Africa, and Central Asia (Yadav & Rai, 2013) for feed and forage purposes. India is the largest producer of pearl millet in the world, with a production of 9.70 Tg from 7.5 million ha (INDIASTAT, 2018). In India, hybrids occupy ~70% (~5.0 million ha) of the area under pearl millet; the rest is under open-pollinated varieties (OPVs) or landraces (Satyavathi, 2017). Since the 1960s, Indian pearl millet breeding programs have harnessed heterosis by developing hybrid cultivars, with productivity increase of 3% per annum during 1990–2017 (Yadav, Singh, Dhillon, & Mohapatra, 2019). In contrast, African pearl millet productivity has not significantly changed during last three decades from 1988 (691 kg ha^{-1}) to 2018 (718 kg ha^{-1}), though area has increased from 15.8 to 22.1 million ha (FAO, 2020). Until recently, African countries grew only conventional landraces and locally adapted, improved OPVs. Their breeding programs have now started focusing on hybrid breeding, yet there is no viable hybrid cultivar in the seed market.

Increasing genetic gain for grain yield requires broadening the genetic base of cultivars by introducing diverse germplasm and use of modern molecular biology tools in order to achieve higher heterosis. The former strategy has been followed in pearl millet hybrid breeding programs in the past to develop a diverse range of productive parental lines for hybrids. This has included the introduction of diverse African germplasm into the Asian breeding programs (Serba, Perumal, Tesso, & Min, 2017; Yadav & Rai, 2013). Later strategy included use of linkage analysis and genome-wide association studies based on different marker systems in detecting quantitative trait loci (QTLs) for important agronomic traits (Serba et al., 2019).

Recent characterization studies based on various agromorphological traits in pearl millet have reported significant variability among both African germplasm (Bashir, Ali, Ali, Melchinger, et al., 2014; Pucher et al., 2015; Sattler et al., 2018) and Asian germplasm (Kumari et al., 2016; Upadhyaya, Reddy, Ramachandran, Kumar, & Ahmed, 2016). Molecular marker-based studies conducted to date have also reported significant diversity among African populations and landraces (Adeoti et al., 2017; Bashir et al., 2015; Diack et al., 2017; Hu et al., 2015; Jika et al., 2017; McBenedict, Chimwamurombe, Kwembeya, & Maggs-Kolling, 2016), and also in Asian landraces (Chowdari, Davierwala, Gupta, Ranjekar, & Govila, 1998; Vom Brocke, Christinck, Weltzien, Presterl, & Geiger, 2003). However, like other crops, the utilization of available germplasm is quite limited in the current pearl millet breeding programs (Serba et al., 2017; Yadav, Bidinger, & Singh, 2009; Yadav et al., 2017). To use the genetic diversity, molecular markers have also been used to assess the utility of marker-based genetic distance (GD) to predict heterosis for grain yield in various crops and found both positive and negative relationships between marker-based GD and yield heterosis (Dias, Picoli, Rocha, & Alfenas, 2004). In pearl millet, Singh et al. (2018) and Singh and Gupta (2019) reported a significant positive correlation between molecular-based GD and better-parent heterosis for grain yield, whereas no such relationship was found in the crosses involving West African OPVs (Sattler et al., 2019).

None of the studies to date has comprehensively analyzed genetic diversity patterns among an extensive collection of both Asian and African origin populations of pearl millet using molecular markers. Therefore, this study investigated Asian and African populations of pearl millet for the extent of diversity and examined the relationship between marker-based GDs and grain-yield heterosis.

Core Ideas

- Significant genetic diversity is available among Asian and African Pearl millet populations.
- Asian and African pearl millet populations grouped irrespective of their geographical origin.
- Higher number of alleles found in African origin-Asian bred populations than other populations.
- No significant correlation found between molecular genetic distance and grain yield heterosis.
- Introgression between African and Asian populations should continue to enhance productivity.

2 | MATERIALS AND METHODS

2.1 | Plant materials and DNA extraction

A series of improved pearl millet populations with genetic materials of African and Asian origins developed by the

pearl millet breeding program of the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) since its inception, in collaboration with its regional partners, were used in this study. A set of 45 pearl millet populations involving 10 Asian origin–Asian bred (abbreviated as As-As), seven African origin–African bred (Af-Af), three (Asian × African) origin–Asian bred [(As × Af)-As], and 25 African origin–Asian bred (Af-As) developed at ICRISAT, Patancheru, were evaluated in this study. Pedigrees of these populations are given in Supplemental Table S1.

For DNA extraction, ~100 seeds of each population were sown in five small plastic pots (10 cm) in a glasshouse, and seedlings (20 per pot) were grown for 12–15 days. Genomic DNA was extracted from leaves of 16 randomly selected individuals per population. A set of 720 DNA samples (45 populations × 16 individuals) was isolated using NucleoSpin 96 Plant II Kit (Macherey-Nagel). Electrophoresis (0.8% agarose gel) was performed to test the quality of DNA and quantify it based on lambda DNA (MBI Fermentas). The working DNA samples were normalized uniformly at a concentration of 10 ng μl^{-1}.

2.2 | Genotyping using SSR markers

Twenty-nine highly polymorphic simple sequence repeat (SSR) markers (Supplemental Table S2), which were mapped earlier across seven pearl millet linkage groups (Allouis, Qi, Lindup, Gale, & Devos, 2000; Budak, Pedraza, Cregan, Baenziger, & Dweikat, 2003; Qi et al., 2004; Rajaram et al., 2013; Senthilvel et al., 2008), were used to genotype the 45 populations. A step-down polymerase chain reaction (PCR) program was performed in a thermal cycler (GeneAmp, PCR System 9700; Applied Biosystems) using 384-well PCR plates. Reaction mixture of 10-µl volume consisted of 2 µl of 10 ng DNA template, 0.5 µl of 1 mM dNTPs, and 0.06 µl of 0.2 U Taq DNA polymerase, 1 µl of 10× Kappa Taq Polymerase buffer with $MgCl_2$, and 1 µl of primer containing 2 pM μl^{-1} of forward and 4 pM μl^{-1} of reverse primer, 0.2 µl of fluorescent dye (either 6-FAM [6-carboxyfluorescein], VIC [2′-chloro-7′phenyl-1,4-dichloro-6-carboxy-fluorescein], NED, or PET). Amplification conditions in the step-down PCR program were an initial denaturation at 94 °C for 5 min, 10 cycles at 94 °C for 25 s, 64 °C (−1 °C per cycle) for 20 s, and 72 °C for 30 s, followed by 37 cycles at 56 °C for 20 s and 72 °C for 30 s, with a final extension of 72 °C for 20 min.

The DNA fragments were size fractioned on an ABI 3700 automatic DNA sequencer (Perkin-Elmer/Applied Biosystems), a fluorescence-based capillary detection system. After confirmation of amplification of 10–15 random samples per locus on agarose gel, PCR products were multiplexed with 1 µl of each dye-labeled product (FAM, VIC, NED, and PET), 7 µl of Hi-Di Formamide (Applied Biosystems), 0.1 µl of the LIZ-labeled (500[-250]) internal size standard, and 3.9 µl of deionized water, centrifuged, and denatured at 94 °C for 2 min. They were immediately cooled in ice and subjected to capillary electrophoresis on ABI 3700 automatic DNA sequencer (Perkin-Elmer/Applied Biosystems). The peaks were sized and the allele calling was done using Gene Mapper 4.0 software (Applied Biosystems, 1998). Check sample Tift $23D_2B_1$ was included during the PCR of each marker and capillary electrophoresis to verify their repeatability.

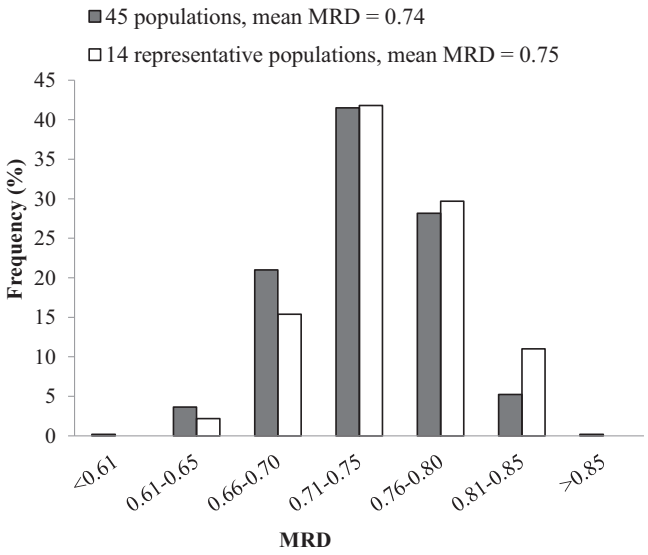

FIGURE 1 Distribution of modified Roger's distance (MRD) of the 45 populations and the 14 representative parental populations based on 29 simple sequence repeat (SSR) markers

2.3 | Assessing association between genetic distance and panmictic heterosis for grain yield

Fourteen parental populations were identified based on the following criteria suggested by Wang, Qiu, Larazo, Angelita, and Xie (2015): (a) representing the original population structure with seven clusters (Table 1), and (b) possessing the maximum allelic variation (representing the distribution of GD of the 45 populations) (Figure 1). Based on the number of populations in each cluster, they were designated as large (>6 populations), medium (6 populations), or small (<6 populations) clusters. Three representative populations from large clusters and one or two representative populations from small to medium clusters were identified. The representative 14 parental populations covered 84.8% of the allelic variation in the

TABLE 1 Distribution of 45 pearl millet populations and 14 representative populations in different clusters based on cluster size and genetic distance (GD)

Cluster no.	No. of populations: Name of the populations				Total	Size of the cluster	Cluster avg. GD	No. and name of the identified representative populations along with avg. GD in parentheses[e]
	As-As[a]	Af-Af[b]	Af-As[c]	(As × Af)-As[d]				
I	4: WRajPop bmr, WRajPop C2, RCB-IC 948, CZ-IC 9802	1: Ugandi	4: AIMP 92901, ICTP 8203, HHVBC, ICMV 155	–	9	Large	0.691	3—Ugandi (0.707), HHVBC (0.675), ICMV 155 (0.675)
II	3: HiTiP 88 C1, CZP-86, Raj 171	2: SOSAT C88, GB 8735	1: ICMP 87703	–	6	Medium	0.704	2—SOSAT C88 (0.691), GB 8735 (0.720)
III	–	3: SC1 C4 bulk, WC C3 bulk, ICMV-IS 92222	2: ICMP 89130, ICMP 96132	1: EC C6	6	Medium	0.698	1—EC C6 (0.703)
IV	2: CZ-IC 618, MRC General bulk	–	4: ICMP 00552, ICMV 94135, Sudan I, ICMV 94132	–	6	Medium	0.697	2—Sudan I (0.695), MRC General bulk (0.704)
V	–	–	5: ICMV 221, Sudan II, WC C-75, JBV 3, GICKV 98771	–	5	Small	0.667	1—GICKV 98771 (0.645)
VI	1: CZ-IC 922	–	3: ICMV 88908, ICMV 93752, ICMP 96201	2: ICMS 7704, ICMP 87307	6	Medium	0.651	2—ICMS 7704 (0.636), ICMV 87307 (0.657)
VII	-	1: NWC C2	6: ICMP 98107, ICMP 97774, ICMP 99901, ICMP 96601, ICMP 97754, ICMP 87237	–	7	Large	0.753	3—ICMP 98107 (0.768), NWC C2 (0.738), ICMP 87237 (0.761)

[a]As-As, Asian origin and Asian bred;
[b]Af-Af, African origin and African bred;
[c]Af-As, African origin and Asian bred;
[d](As × Af)-As, (Asian × African) origin and Asian bred;
[e]Mean GD of the population in relation to rest of the populations in the respective cluster.

45 populations. Modified Roger's distance (MRD) among the 14 representative populations ranged from 0.64 to 0.85, with a mean of 0.75, representing 45 populations (mean of 0.74 and range from 0.60 to 0.86).

The 14 representative populations were crossed according to full diallel mating design at ICRISAT during the 2016 summer season (February to May). At least 15–20 panicles from each parent were pollinated with bulk pollen collected from 20–25 plants of the respective crossing parent. The 14-parent diallel cross yielded 182 population hybrids (91 F_1s and 91 reciprocal F_1s). To generate enough quantity of seed for multilocation trials, equal quantities of seeds of each F_1 and its reciprocal cross were bulked together, making the total number of crosses 91. The hybrids were evaluated along with parental populations and two popular OPV checks (ICTP 8203 and Dhanashakti) in an α-lattice design with two replications. The evaluation was conducted at three locations: ICRISAT, Patancheru, Telangana (17°30′ N, 78°27′ E, 545 m asl); Regional Agricultural Research Station, Palem, Telangana (16°53′ N, 78°23′ E, 545 m asl); and Pearl Millet Research Station, Junagadh Agricultural University, Jamnagar, Rajasthan (22°28′ N, 70°04′ E and 27.6 m asl), during the rainy season (June–October) of 2016. At Patancheru, each entry was planted in four rows of 4-m length (planted on 6 June), with an inter-row spacing of 75 cm and an intra-row spacing of 15 cm. At Palem (planted on 22 June) and Jamnagar (planted on 25 July), each entry was planted in four rows of 4-m length, with an inter-row spacing of 60 cm and an intra-row spacing of 12 cm. All the recommended agronomic practices were followed at all locations to obtain good crop growth. There were no major incidences of diseases and pests. All the panicles were harvested at physiological maturity (on 14 September at ICRISAT, 29 September at Palem, and 31 October 2016 at Jamnagar). The harvested material was sun dried for 10–15 d and threshed, and data on grain yield (kg ha^{-1} adjusted to 125 g kg^{-1} moisture) were recorded.

2.4 | Statistical data analysis

2.4.1 | Diversity analysis

Diversity analysis was conducted using 29 SSR markers to obtain summary statistics, which included allelic richness, as determined by the total number of alleles detected; alleles per locus; major allele frequency; gene diversity; and heterozygosity. Summary statistics for populations (individually and origin-wise) were estimated using PowerMarker version 3.25 (Liu & Muse, 2005). The breeding populations were assumed to be in Hardy–Weinberg equilibrium (HWE) since they were bred and maintained at ICRISAT through random mating. The genetic dissimilarities in each pair of populations based on the MRD matrix was computed according to Wright (1978) using SAS version 9.4 (SAS Institute, 2017). A cluster diagram was constructed based on the neighbor-joining (NJ) method using the DARwin 5.0 program (Perrier & Jacquemoud-Collet, 2006). Analysis of molecular variance (AMOVA) was performed according to Michalakis and Excoffier (1996) to divide molecular genetic variance into components attributed to variance between and within populations. The pairwise fixation index (F_{ST}) method was used to infer the distinctness of clusters in a neighbor-joining tree. The analysis was carried out using R program statistical software (R Core Team, 2017). The test to determine deviations from HWE and the existence of nonrandom associations of populations across all SSR loci was computed according to Guo and Thompson (1992) using PowerMarker version 3.25 (Liu & Muse, 2005).

The model-based software program STRUCTURE 2.3 was used to infer population structure by Bayesian approach using SSR marker data (Pritchard, Stephens, & Donnelly, 2000). The optimal value of K (the number of clusters) was determined by performing five independent runs with $K = 2$–10. The length of burn-in of the Markov chain Monte Carlo (MCMC) interactions was set to 100,000, and data were collected across 100,000 MCMC interactions in each run. The optimal value of K was computed using both the ad hoc procedure introduced by Pritchard et al. (2000) and the method developed by Evanno, Regnaut, and Goudet (2005), which improves clustering of closely related populations. The final K value was determined based on (a) the rate of change in mean log probability of the data [ln$P(D)$] between successive K, (b) the stability of grouping pattern across the runs, and (c) the germplasm information on the examined material.

2.4.2 | Analysis of variance

Combined ANOVA was performed following the mixed model method of restricted maximum likelihood (REML) using the PROC MIXED procedure in SAS version 9.4 (SAS Institute, 2017), considering locations, genotypes, and replications as fixed effects and blocks as a random effect. Individual location variances were modeled to error distribution using repeated statement in SAS mixed procedure. Midparent heterosis was calculated as panmictic midparent heterosis (PMPH) according to Lamkey and Edwards (1999), and commercial heterosis was calculated as panmictic commercial heterosis (PCH). The PMPH and PCH were estimated using the following formulae:

$$\text{PMPH}(\%) = \frac{\overline{\text{HP}} - \overline{\text{MP}}}{\overline{\text{MP}}} \times 100$$

$$\text{PCH}(\%) = \frac{\overline{\text{HP}} - \overline{\text{CH}}}{\overline{\text{CH}}} \times 100$$

where $\overline{\text{HP}}$ is hybrid performance or mean grain yield of the F_1 population hybrid; $\overline{\text{MP}}$ is mean grain yield of the two parental populations involved in a cross = (P1 + P2)/2; P1 and P2 are grain yields of parental Populations 1 and 2, respectively; and $\overline{\text{CH}}$ is mean grain yield of the commercial check.

ICTP 8203 was used as an OPV check to calculate PCH since it is widely cultivated in India and has desirable traits, such as early maturity, large seed size, resistance to downy mildew (*Sclerospora graminicola*), and good yielding ability in drought-affected environments. Pearson correlation coefficients (r) were calculated and significance tests were performed between MRD GD and PMPH using PROC CORR program in SAS version 9.4 (SAS Institute, 2017).

3 | RESULTS AND DISCUSSION

3.1 | Marker-based genetic variation in pearl millet populations

The 29 SSR loci detected 435 alleles in 720 samples (16 individuals randomly sampled from each of the 45 populations). The number of alleles per locus ranged from 6 (*Xicmp*3032) to 32 (*Xpsmp*2070), with a mean of 15 alleles per locus, indicating a high level of molecular diversity. Gene diversity (H_e) varied from 0.57 (*Xipes*0082) to 0.96 (*Xpsmp*2079.2), with a mean of 0.75. Observed heterozygosity (H_o) ranged from 0.10 (*Xpsmp*2070) to 0.67 (*Xicmp*3088), with a mean of 0.31, indicating a high level of heterozygosity within the populations (Table 2).

Among the 45 populations, the number of alleles ranged from 4.28 (SOSAT C88) to 6.07 (ICMV 97754), H_e ranged from 0.57 (SOSAT C88) to 0.70 (Sudan I), H_o ranged from 0.22 (GICKV 98771) to 0.41 (RCB-IC 948), and inbreeding coefficient ranged from 0.39 (RCB-IC 948) to 0.66 (GICKV 98771) (Supplemental Table S3). Among the four categories of populations, the average number of alleles per marker ranged from 8.24 [(As × Af)-As] to 13.72 (Af-As). However, H_e, H_o, and inbreeding coefficient showed comparable values among the four categories of populations (Table 3).

The relatively high gene diversity ($H_e = 0.75$), observed heterozygosity ($H_o = 0.31$), and mean number of alleles per locus (15 alleles), obtained in the current study, indicated the presence of considerable genetic variability among the 45 pearl millet populations under investigation. The present study detected a higher number of alleles and gene diversity than found in previous studies using SSRs in African pearl millet accessions (Adeoti et al., 2017; Diack et al., 2017). This might be due to use of more markers in our study and sampling of a larger number of individuals per population. These highly diverse populations can serve as promising sources to broaden the genetic base of the current pearl millet breeding materials if used strategically, based on trait adaptation and specific needs of the regions. Germplasm with high genetic diversity can also be used to develop segregating populations, in association mapping studies, and can provide enriched gene resources for allele mining in the grass family (Wang et al., 2012).

3.2 | Genetic analysis of populations

The HWE assumes a random mating system that can be assessed by computing the deviation of the H_o from an unbiased estimate of H_e. The deviation from HWE was highly significant for all 29 SSR loci ($p < .001$). Populations achieve HWE proportions after one generation of random mating if there is no selection, mutation, or migration (Hardy, 1908; Weinberg, 1909). Given the breeding and maintenance procedures of pearl millet populations at ICRISAT (populations are random mated in isolation during their development and multiplication process), it was expected that the populations were in HWE. However, all 29 SSR loci used in the present study indicated deviation from HWE. This deviation has also been reported in previous studies in other highly cross-pollinated crops, such as maize (Qi-Lun, Ping, Ke-Cheng, & Guang-Tang, 2008; Reif et al., 2004). This could mainly be attributed to genetic causes, such as nonrandom mating, artificial subgrouping of individuals within populations, selection favoring homozygotes, and experimental errors during laboratory assays. Reif et al. (2004) mentioned that experimental errors could probably be the major cause of heterozygote deficiency within populations, apart from genuine genetic causes.

3.3 | Analysis of molecular variance

Results of AMOVA revealed that the variance within populations was significantly higher (88.94%) than that found among the populations (11.07%). The F_{ST} value (0.11) indicated a moderate level of genetic differentiation among the populations, as it accounted for 11% of the total genetic variation. The intrapopulation genetic variation was found higher than many other studies conducted earlier on different pearl millet populations and landraces derived from different regions of Africa (Bashir et al., 2015; Stich et al., 2010; Dussert, Snirc, and Robert, 2015), and also on Indian landraces (Vom Brocke et al., 2003).

TABLE 2 Allelic composition, gene diversity, and heterozygosity of the 29 simple sequence repeat (SSR) loci in 45 pearl millet populations

SSR loci	Allele size range bp	No. of alleles	Major allele frequency	Gene diversity (H_e)	Heterozygosity (H_o)
Xctm10	183–219	19	0.19	0.89	0.15
Xicmp3032	202–217	6	0.43	0.66	0.35
Xicmp3043	198–228	10	0.46	0.66	0.45
Xicmp3088	153–192	13	0.24	0.85	0.67
Xipes0082	154–209	10	0.58	0.57	0.42
Xipes0152.2	109–137	14	0.40	0.77	0.31
Xipes0186	169–293	11	0.40	0.70	0.56
Xipes0200	174–210	11	0.35	0.78	0.33
Xipes0203	229–307	27	0.22	0.87	0.47
Xipes0213	163–181	7	0.44	0.62	0.23
Xipes0220.1	172–216	18	0.33	0.78	0.33
Xipes0236	215–263	15	0.36	0.74	0.17
Xpsmp2030	102–160	21	0.48	0.72	0.32
Xpsmp2068	112–162	23	0.15	0.89	0.24
Xpsmp2070	204–278	32	0.13	0.93	0.1
Xpsmp2079.2	117–179	31	0.07	0.96	0.2
Xpsmp2085	181–199	9	0.39	0.71	0.33
Xpsmp2086	107–149	18	0.50	0.71	0.23
Xpsmp2089	117–159	21	0.16	0.90	0.32
Xpsmp2090	189–211	12	0.32	0.77	0.28
Xpsmp2201	363–385	9	0.56	0.64	0.14
Xpsmp2202	161–185	13	0.61	0.59	0.39
Xpsmp2203	351–389	15	0.21	0.85	0.37
Xpsmp2207	315–333	10	0.59	0.61	0.37
Xpsmp2220	136–156	11	0.41	0.74	0.44
Xpsmp2232	247–275	11	0.28	0.78	0.2
Xpsmp2248	177–209	17	0.35	0.83	0.2
Xpsmp2249	154–182	9	0.61	0.58	0.19
Xpsmp2275	267–300	12	0.37	0.78	0.27
Total		435			
Mean		15	0.37	0.75	0.31

These results indicated the presence of sufficient variation at the population level in the materials involved in this study, which can be used to select parents to generate new synthetic populations with good adaptation and yield, as suggested by Hartings et al. (2008).

3.4 | Genetic distance, cluster analysis, and population structure analysis

The highest GD (0.86) was found between Sudan II and ICMP 97774 and the lowest GD (0.56) was found between ICMP 96201 and CZ-IC 922. Cluster analysis, based on the genetic dissimilarity matrix, grouped 45 pearl millet populations into seven major clusters (Figure 2). All the clusters showed significant F_{ST} values, indicating their distinctness (Supplemental Table S4). The number of populations in each cluster varied from five to nine. The As-As populations were distributed across four clusters; Cluster I (4 populations), Cluster II (3 populations), Cluster IV (2 populations), and Cluster VI (1 population). Similarly, Af-Af populations were found distributed across four clusters: Cluster I (1 population), Cluster II (2 populations), Cluster III (3 populations), and Cluster VII

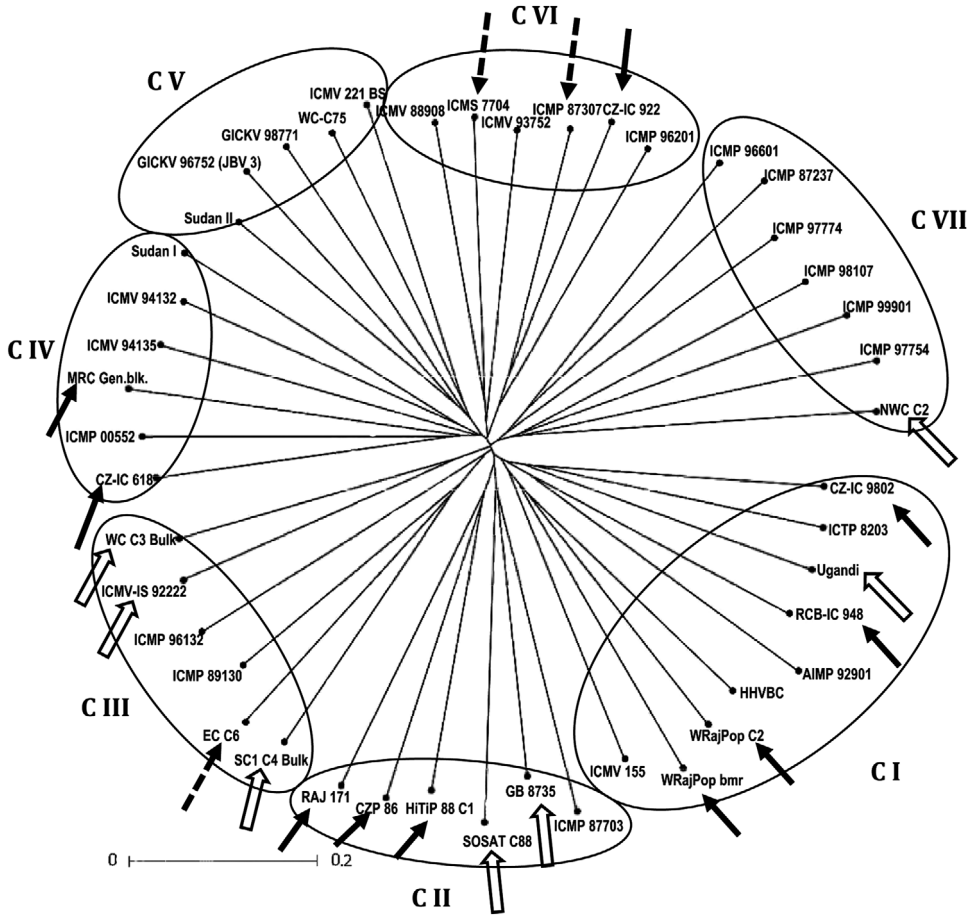

FIGURE 2 Unweighted neighbor-joining tree of 45 pearl millet populations (10 As-As [Asian origin and Asian bred], 7 Af-Af [African origin and African bred], 3 (As × Af)-As [(Asian × African) origin and Asian bred], and 25 Af-As [African origin and Asian bred]) based on modified Roger's distance matrix using simple sequence repeat (SSR) markers. The filled arrows denote As-As populations, the unfilled arrows denote Af-Af populations, the dotted arrows denote (As × Af)-As populations, and the rest are Af-As populations. CI–CVII denote Clusters I–VII

(1 population). However, Clusters IV, V, VI, and VII were predominantly composed of Af-As populations, with very little intermixing of As-As and Af-Af populations.

The population–structure analysis revealed that all 45 populations could be delineated into three main groups at $K = 3$ (Figure 3) on the basis of the rate of change in $\ln P(D)$. Group 1 consisted of 24 populations (53.3%), Group 2 had nine populations (20%), and Group 3 had the remaining 12 populations (26.7%). Group 1 was dominated by 14 Af-As populations (58.3%), four As-As (16.7%) populations, and three populations each of (As × Af)-As (12.5%) and Af-Af (12.5%) populations. Among the nine populations of Group 2, eight (88.9%) were Af-As populations, and one (11.1%) was an Af-Af population. Group 3 had an intermix of six As-As (50%), three Af-Af (25%), and three Af-As (25%) populations. Among the 25 Af-As populations, 22 (88%) were found to be together in the first two groups; out of 10 As-As populations, six (60%) were found to be in Group 3, and four (40%) were found to be in Group 1, whereas seven Af-Af populations were found to be distributed across these groups, with three populations each in Groups 1 and 3.

Both MRD matrix-based cluster analysis and population structure analysis showed comparable results for grouping populations. Both analyses revealed a separate clustering of a majority of Af-As populations but did not reveal a clear-cut distinction between As-As and Af-Af populations. Pearl millet populations within Clusters IV, V, VI, and VII (mostly Af-As populations) were found together in Groups 1 and 2 according to the structure analysis. Similarly, populations in Cluster I (As-As and Af-As) belonged to Group 3. Overall, the results showed poor differentiation of populations (As-As and Af-Af) from different geographical (Asian and African) regions which are supported by results of previous studies reporting the absence of any specific structuration among pearl millet accessions from different regions of West Africa (Adeoti et al., 2017) and among landraces of different regions of Sudan and West Africa (Bashir et al., 2015). Based on the

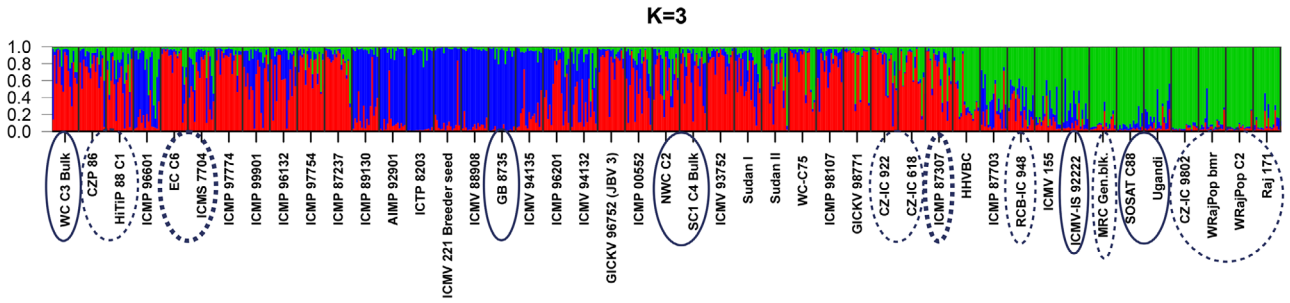

FIGURE 3 Population structure analysis showing the grouping of 45 pearl millet populations. The color bands represent different groups: red denotes Group 1, blue denotes Group 2, and green denotes Group 3. The populations are denoted by circles: lined circle = As-As (Asian origin and Asian bred); full circle = Af-Af (African origin and African bred); thick dotted circle = (As × Af)-As {(Asian × African) origin and Asian bred}; and the uncircled ones = Af-As (African origin and Asian bred). *K*, number of clusters

clustering pattern, it can be inferred that the molecular GD between the 45 African and Asian populations did not show their clear grouping by geographical origin. This may be attributed to the evolutionary process and the manner in which dispersal of pearl millet from the African continent occurred across India during the domestication process (Fuller & Boivin, 2009; Manning, Pelling, Higham, Schwenniger, & Fuller, 2011), and also because of the spread of germplasm from West Africa to Asian countries, leading to vast gene exchange among the gene pools, resulting in reduced nucleotide diversity (Hu et al., 2015).

Some of the breeding populations related by parentage, or bred for the same region, were found in same clusters, indicating some genetic relatedness; for example, in Cluster II, three As-As populations bred using germplasm from Rajasthan (a northwestern state of India that grows 50% of pearl millet in India) grouped together, and these were found to be grouped separately from three Af-Af populations, which were bred using West African germplasm. Cluster III had a majority of African origin populations from Uganda and Nigeria, except for EC C6, which was an (As × Af)-As population. Cluster IV was composed of four Af-As populations and two As-As populations (CZ-IC 618 and MRC general bulk). Of the four Af-As populations, ICMV 94135 and ICMV 94132 were derived from the Large Grain Populations (LaGraP), whereas Sudan I and ICMP 00552 were bred using materials originating in Senegal. The two As-As populations were developed using Mandor Restorer Composite (MRC), a composite developed with 12 breeding lines adapted to arid environment such as drought-prone environments of western Rajasthan (Yadav et al., 2012). Also, Cluster V contained five populations belonging to the Af-As populations. Two of these populations (GICKV 98771 and GICKV 96752) were derived from smut-resistant composite (SRC) of African origin. Cluster VI was composed of six populations, which included three Asian adaptation- and origin-linked populations. Two of these populations (ICMS 7704 and ICMP 87307) were developed in Asia through Asian × African crosses, and the third As-As population (CZ-IC 922) was developed at Jodhpur (Rajasthan) using EC C6 (African origin). The other three populations in Cluster VI were derived from African origin genetic material (e.g., SRC [ICMV 93752 and ICMP 96201]) and a bold-seeded early composite (ICMV 88908). Also, Cluster VII consisted of seven populations of Af-As, three of which had SRC in their pedigree (ICMP 98107, ICMP 97774, and ICMP 97754), and four had different genetic backgrounds developed using materials with African parentage: Uganda (ICMP 87237), Zimbabwe (ICMP 96601), Nigeria (NWC C2), and half-sib progenies of African origin material HHVBC (ICMP 99901). These instances of genetically related populations found in common clusters indicated that markers used in this study were able to differentiate populations sharing common gene pools.

The overall grouping pattern of 45 populations showed that Af-As populations clustered separately from As-As and Af-Af populations. This might have happened because a diverse range of African germplasm sources was used during the early phase of the Indian pearl millet improvement program to develop several gene pools and composites (e.g., early composite, medium composite, late composite, smut-resistant composite, high-tillering composite, bold-seeded composite, dwarf composite, and high head-volume composite). Selection among these gene pools yielded promising Af-As OPVs, such as WC-C75, ICMV 155, ICTP 8203, ICMV 221, JBV 3, and CZP 9802, which are adapted to Indian conditions and were found to be grouped together. This use of breeding materials of African origin in Asian (Indian) pearl millet improvement programs led to the fixation of alleles favoring adaptation to Asian regions. Serba et al. (2019) also reported that West African genetic accessions have been used extensively in Indian breeding programs. This seems to have led to the differentiation of Af-As populations from the As-As and Af-Af populations, as observed in the present study.

TABLE 3 Genetic diversity in 45 pearl millet populations of different origins, assessed by 29 simple sequence repeat (SSR) markers

Population category[a]	No. of populations	Total no. of alleles	Avg. number of alleles	Gene diversity (H_e)	Heterozygosity (H_o)	Inbreeding coefficient (f)
As-As	10	359	11.41	0.73	0.33	0.56
Af-Af	7	366	11.69	0.75	0.32	0.57
(As × Af)-As	3	265	8.24	0.71	0.30	0.59
Af-As	25	427	13.72	0.75	0.30	0.60

[a]As-As, Asian origin and Asian bred; Af-Af, African origin and African bred; Af-As, African origin and Asian bred; (As × Af)-As, (Asian × African) origin and Asian bred.

TABLE 4 Analysis of variance of population hybrids for grain yield across three locations

Source of variation	df	*F* value
Locations	2	109.65***
Replications (Loc)	3	3.39*
Genotypes	104	4.70***
Hybrids (Hyb)	90	4.05***
Parents (Par)	13	9.26***
Hyb vs. Par	1	4.54*
Locations × genotypes	208	2.23***
Loc × Hyb	180	2.18***
Loc × Par	26	2.58***
Loc × (Hyb vs. Par)	2	2.22
Covariance parameters	Estimate	
Block (Loc** × Rep)	19,599**	
Error at Patancheru	54,037	
Error at Palem	85,109	
Error at Jamnagar	72,303	
Pooled error	70,401	

*, **, ***Significant at the .05, .01, and .001 probability levels, respectively.

The genetic divergence of Af-As populations from As-As and Af-Af was further evidenced by the larger number of alleles per locus (13.7) observed in Af-As populations compared with As-As (11.6) and Af-Af (11.4) populations. Such allelic differences between interregional populations (Af-As) and intraregional populations (As-As and Af-Af) suggested that breeding efforts involving interregional germplasm to develop improved cultivars and germplasm with a higher number of adaptive alleles should continue.

3.5 | Grain yield of population hybrids, panmictic midparent heterosis, and its association with genetic distance

Analysis of variance for grain yield revealed highly significant variance attributable to locations (environments), indicating that the materials were evaluated under diverse environments (Table 4). Large and significant genotypic variation observed in population hybrids and parental populations indicated the existence of adequate genetic variation in parental populations and their hybrids. Significant "hybrids vs. parents" variance indicated the presence of significant heterosis for grain yield in hybrids. Location × hybrid and location × parent interactions were highly significant, suggesting that different genotypes (hybrids or parental populations) had differential response at different test locations.

Across locations, grain yields of population hybrids ranged from 1,652 (EC C6 × GB 8735) to 2,992 kg ha^{-1}

(GB 8735 × ICMP 87307), and grain yields of parental populations ranged from 1,322 (ICMS 7704) to 2,667 kg ha^{-1} (Sudan I). Across locations, PMPH varied from −21.7 (EC C6 × ICMP 98107) to 62.1% (ICMS 7704 × ICMV 155), with a mean of 6.4%, and 12 population hybrids had significant positive PMPH (Supplemental Table S5). Hybrid yield advantage over OPV check ICTP 8203 varied from −3.9 (EC C6 × GB 8735) to 74.0% (GB 8735 × ICMP 87307), with a mean of 30.6% across locations. Out of 91 hybrids, 60 showed >25% heterosis over OPV check ICTP 8203 (Supplemental Table S5). More than half of the population hybrids investigated in this study had significant positive and higher heterosis compared with the standard check ICTP 8203. This clearly demonstrated that the performance of such hybrids could be significantly higher than that of the commercial check, making them a highly suitable base material for further crop improvement. Earlier, Yadav et al. (2012) reported significant heterosis for grain yield while evaluating crosses between Indian and African composites. The highest-yielding population hybrid (GB 8735 × ICMP 87307) had the highest PCH of 74.0%, followed by hybrids Sudan I × Ugandi and HHVBC × ICMP 87307 with ~50% yield heterosis. All these high heterotic cross combinations can be used to improve combining ability and to develop high-yielding, improved populations adapted to regions where OPVs are cultivated. They can also be improved for combining ability and used as base parental populations to derive superior inbreds for hybrid breeding, following reciprocal recurrent selection.

The correlation coefficient between GD of parental populations and PMPH of these 91 population hybrids was investigated. When all the hybrids were partitioned into three groups with high, moderate, and low GD within each group, the correlation coefficient between GD and PMPH was nonsignificant: r was −.24 ($p < .26$) in 26 hybrids with high GD (0.78–0.86); r was −.13 ($p < .39$) in 44 hybrids with moderate GD (0.69–0.77); and r was −.09 ($p < .68$) in 21 hybrids with low GD (0.60–0.68). This indicated that prediction of grain yield heterosis based on GD calculated using molecular markers was not possible in pearl millet populations. Earlier, Dias et al. (2004) reviewed the association of GD with hybrid performance in several crops in 54 different studies and reported that 28 studies showed a positive correlation, whereas the remaining 26 showed a negative correlation or inconclusive results. Also, studies in pearl millet by Chowdari et al. (1998) using RAPD (random amplified polymorphic DNA) markers and by Gupta et al. (2018) using SSR markers did not find a significant correlation between marker-based GDs and midparent heterosis for yield. In the case of West African pearl millet populations, Sattler et al. (2019) also did not find a significant correlation between SSR-based MRD and PMPH. On the contrary, one of the studies in pearl millet (Singh & Gupta, 2019) and a few other studies in maize (Reif et al., 2003a, 2003b) found a significant positive association between GD and yield heterosis. Our study used a set of neutral markers, though non-neutral markers could have helped in finding a relationship between GD and PMPH more accurately if the markers were linked to yield-related QTLs, as earlier suggested by several workers (Bashir et al., 2015; Jordan et al., 2003; Zhang, Gao, Saghai-Maroof, Yang, & Li, 1995). Hence, it is necessary that genetic diversity should be investigated using a higher number of markers that are evenly distributed across the genome and the markers linked to the yield-related traits may help to predict grain yield heterosis. The results suggested the need to generate detailed information on heterotic pools among diverse populations originating and bred in Africa and Asia to enable breeders to use such materials to develop a new series of improved cultivars.

4 | CONCLUSIONS

The present study on SSR marker-based genetic diversity showed the presence of significant genetic variation among African and Asian pearl millet populations. Populations of Af-As group formed clusters separate from those formed by As-As or Af-Af populations. This showed that targeted breeding efforts made in the past had led to the development of new gene pools adapted to the Asian region because of the introgression of materials of Asian and African origin. This introgression of African gene pools into Asian pearl millet breeding pools has contributed significantly to the enhancement of pearl millet productivity in India, which should be continued. No correlation between marker-based GD and heterosis for grain yield indicated that heterosis could not be predicted on the basis of GD. Therefore, in addition to GD between the parents, combining ability patterns also need to be assessed for better prediction of heterosis. This study also underlined the need to further investigate the existence of heterotic groups among pearl millet populations to identify heterotic populations to develop high-yielding OPVs or to derive superior inbreds for hybrid development. The study also identified some parental combinations that can further be used for the development of superior populations and inbreds in pearl millet cultivar development programs.

ACKNOWLEDGMENTS

This research was financially supported by the CGIAR Research Program on Dryland Cereals Scholarship (CRP-DC) and the S.M. Sehgal Foundation Fund (USA) for carrying out a doctoral study of K. Sudarshan Patil at ICRISAT. We gratefully acknowledge Dr. C. T. Hash (formerly Principal Scientist, ICRISAT, Niger) for helping with

pedigrees of populations involved in the study. Thanks are also due to Dr. Marilyn L. Warburton (USDA-ARS Corn Host Plant Resistance Research Unit, PO Box 9555, Mississippi State) for her valuable inputs and suggestions in planning the molecular diversity work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ORCID

K. Sudarshan Patil https://orcid.org/0000-0002-8218-2674

REFERENCES

Adeoti, K., Djedatin, G., Ewedje, E., Beule, T., Santoni, S., Rival, A., & Jaligot, E. (2017). Assessment of genetic diversity among cultivated pearl millet (*Pennisetum glaucum*, Poaceae) accessions from Benin, West Africa. *African Journal of Biotechnology*, *16*, 782–790. https://doi.org/10.5897/ajb2017.15898

Allouis, S., Qi, X., Lindup, S., Gale, M. D., & Devos, K. M. (2000). Construction of a BAC library of pearl millet, *Pennisetum glaucum*. *Theoretical and Applied Genetics*, *102*, 1200–1205. https://doi.org/10.1007/s001220100559

Applied Biosystems. (1998). Genotyper 3.1. Foster City, CA: Applied Biosystems.

Bashir, E. M. A., Ali, A. M., Ali, A. M., Melchinger, A. E., Parzies, H. K., & Haussmann, B. I. G. (2014). Characterization of Sudanese pearl millet germplasm for agro-morphological traits and grain nutritional values. *Plant Genetic Resources*, *12*, 35–47. https://doi.org/10.1017/S1479262113000233

Bashir, E. M. A., Ali, A. M., Ali, A. M., Mohamed, E. T. I., Melchinger, A. E., Parzies, H. K., & Haussmann, B. I. G. (2015). Genetic diversity of Sudanese pearl millet (*Pennisetum glaucum* (L.) R. Br.) landraces as revealed by SSR markers, and relationship between genetic and agro-morphological diversity. *Genetic Resources and Crop Evolution*, *62*, 579–591. https://doi.org/10.1007/s10722-014-0183-5

Budak, H., Pedraza, F., Cregan, P. B., Baenziger, P. S., & Dweikat, I. (2003). Development and utilization of SSRs to estimate the degree of genetic relationships in a collection of pearl millet germplasm. *Crop Science*, *43*, 2284–2290. https://doi.org/10.2135/cropsci2003.2284

Chowdari, K. V., Davierwala, A. P., Gupta, V. S., Ranjekar, P. K., & Govila, O. P. (1998). Genotype identification and assessment of genetic relationships in pearl millet [*Pennisetum glaucum* (L.) R. Br] using microsatellites and RAPDs. *Theoretical and Applied Genetics*, *97*, 154–162. https://doi.org/10.1007/s001220050880

De Assis, R. L., De Freitas, R. S., & Mason, S. C. (2018). Pearl millet production practices in Brazil: A review. *Experimental Agriculture*, *54*, 699–718. https://doi.org/10.1017/S0014479717000333

Diack, O., Kane, N. A., Berthouly-Salazar, C., Gueye, M. C., Diop, B. M., Fofana, A., … Bernaud, A. (2017). New genetic insights into pearl millet diversity as revealed by characterization of early- and late-flowering landraces from Senegal. *Frontiers in Plant Science*, *8*. https://doi.org/10.3389/fpls.2017.00818

Dias, L. A. d. S., Picoli, E. A. d. T., Rocha, R. B., & Alfenas, A. C. (2004). A priori choice of hybrid parents in plants. *Genetics and Molecular Research*, *3*, 356–368.

Dussert, Y., Snirc, A., & Robert, T. (2015). Inference of domestication history and differentiation between early- and late-flowering varieties in pearl millet. *Molecular Ecology*, *24*, 1387–1402. https://doi.org/10.1111/mec.13119

Evanno, G., Regnaut, S., & Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: A simulation study. *Molecular Ecology*, *14*, 2611–2620. https://doi.org/10.1111/j.1365-294X.2005.02553.x

FAO. (2020). *FAO statistical database*. Rome: FAO. Retrieved from http://www.fao.org/faostat/en/#data/QC

Fuller, D. Q., & Boivin, N. L. (2009). Crops, cattle and commensals across the Indian Ocean: Current and potential archaeobiological evidence. *Études Océan Indien*, 42–43, 13–46. https://doi.org/10.4000/oceanindien.698

Guo, S. W., & Thompson, E. A. (1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics*, *48*, 361–372. https://doi.org/10.2307/2532296

Gupta, S. K., Nepolean, T., Shaikh, C. G., Rai, K. N., Hash, C. T., Das, R. R., & Rathore, A. (2018). Phenotypic and molecular diversity-based prediction of heterosis in pearl millet (*Pennisetum glaucum* L.(R.) Br.). *The Crop Journal*, *6*, 271–281. https://doi.org/10.1016/j.cj.2017.09.008

Hardy, G. H. (1908). Mendelian proportions in a mixed population. *Science*, *28*, 49–50. http://doi.org/10.1126/science.28.706.49

Hartings, H., Berardo, N., Mazzinelli, G. F., Valoti, P., Verderio, A., & Motto, M. (2008). Assessment of genetic diversity and relationships among maize (*Zea mays* L.) Italian landraces by morphological traits and AFLP profiling. *Theoretical and Applied Genetics*, *117*, 831–842. https://doi.org/10.1007/s00122-008-0823-2

Hu, Z., Mbacké, B., Perumal, R., Guèye, M. C., Sy, O., Bouchet, S., … Morris, G. P. (2015). Population genomics of pearl millet (*Pennisetum glaucum* (L.) R. Br.): Comparative analysis of global accessions and Senegalese landraces. *BMC Genomics*, *16*(1). https://doi.org/10.1186/s12864-015-2255-0

INDIASTAT. (2018). *INDIASTAT database*. Datanet India. Retrieved from https://www.indiastat.com/table/agriculture-data/2/bajra-spiked-millet/17198/7267/data.aspx

Jika, A. N., Dussert, Y., Raimond, C., Garine, E., Luxereau, A., Takvorian, N., … Robert, T. (2017). Unexpected pattern of pearl millet genetic diversity among ethno-linguistic groups in the Lake Chad basin. *Heredity*, *118*, 491–502. https://doi.org/10.1038/hdy.2016.128

Jordan, D. R., Tao, Y., Godwin, I. D., Henzell, R. G., Cooper, M., & McIntyre, C. (2003). Prediction of hybrid performance in grain sorghum using RFLP markers. *Theoretical and Applied Genetics*, *106*, 559–567. https://doi.org/10.1007/s00122-002-1144-5

Kumari, J., Bag, M. K., Pandey, S., Jha, S. K., Chauhan, S. S., Jha, G. K., … Dutta, M. (2016). Assessment of phenotypic diversity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] germplasm of Indian origin and identification of trait-specific germplasm. *Crop Pasture Science*, *67*, 1223–1234. https://doi.org/10.1071/CP16300

Lamkey, K. R., & Edwards, J. W. (1999). Quantitative genetics of heterosis. In J. G. Coors & S. Pandey (Eds.), *The genetics and exploitation of heterosis in crops* (pp. 31–48). Madison, WI: ASA and CSSA. https://doi.org/10.2134/1999.geneticsandexploitation.c4

Liu, K., & Muse, S. V. (2005). Power Marker: An integrated analysis environment for genetic marker analysis. *Bioinformatics*, *21*, 2128–2129. https://doi.org/10.1093/bioinformatics/bti282

Manning, K., Pelling, R., Higham, T., Schwenniger, J., & Fuller, D. Q. (2011). 4500-year old domesticated pearl millet (*Pennisetum glaucum*) from the Tilemsi valley, Mali: New insights into an alternative cereal domestication pathway. *Journal of Archaeological Science*, *38*, 312–322. https://doi.org/10.1016/j.jas.2010.09.007

McBenedict, B., Chimwamurombe, P., Kwembeya, E., & Maggs-Kolling, G. (2016). Genetic diversity of Namibian *Pennisetum glaucum* (L.) R. Br. (pearl millet) landraces analyzed by SSR and morphological markers. *Scientific World Journal*, *2016*. https://doi.org/10.1155/2016/1439739

Michalakis, Y., & Excoffier, L. (1996). A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. *Genetics*, *142*, 1061–1064.

Perrier, X., & Jacquemoud-Collet, J. P. (2006). DARwin software. CIRAD. Retrieved from http://darwin.cirad.fr/darwin

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*, 945–959.

Pucher, A., Sy, O., Angarawai, I. I., Gondah, J., Zangre, R., Ouedraogo, M., … Haussmann, B. I. G. (2015). Agro-morphological characterization of West and Central African pearl millet accessions. *Crop Science*, *55*, 737–748. https://doi.org/10.2135/cropsci2014.06.0450

Qi, X., Pittaway, T. S., Lindup, S., Liu, H., Waterman, E., Padi, F. K., … Devos, K. M. (2004). An integrated genetic map and a new set of simple sequence repeat markers for pearl millet (*Pennisetum glaucum*). *Theoretical and Applied Genetics*, *109*, 1485–1493. https://doi.org/10.1007/s00122-004-1765-y

Qi-Lun, Y., Ping, F., Ke-Cheng, K., & Guang-Tang, P. (2008). Genetic diversity based on SSR markers in maize (*Zea mays* L.) landraces from Wuling mountain region in China. *Journal of Genetics*, *87*, 287–291. https://doi.org/10.1007/s12041-008-0046-y

R Core Team. (2017). *R: A language and environment for statistical computing*. Vienna: R Foundation for Statistical Computing.

Rajaram, V., Nepolean, T., Senthilvel, S., Varshney, R. K., Vadez, V., Srivastava, R. K., … Hash, C. T. (2013). Pearl millet [*Pennisetum glaucum* (L.) R. Br.] consensus linkage map constructed using four RIL mapping populations and newly developed EST-SSRs. *BMC Genomics*, *14*(1). https://doi.org/10.1186/1471-2164-14-159

Reif, J. C., Melchinger, A. E., Xia, X. C., Warburton, M. L., Hoisington, D. A., Vasal, S. K., … Frisch, M. (2003a). Genetic distance based on simple sequence repeats and heterosis in tropical maize populations. *Crop Science*, *43*, 1275–1282. https://doi.org/10.2135/cropsci2003.1275

Reif, J. C., Melchinger, A. E., Xia, X. C., Warburton, M. L., Hoisington, D. A., Vasal, S. K., … Frisch, M. (2003b). Use of SSRs for establishing heterotic groups in subtropical maize. *Theoretical and Applied Genetics*, *107*, 947–957. https://doi.org/10.1007/s00122-003-1333-x

Reif, J. C., Xia, X. C., Melchinger, A. E., Warburton, M. L., Hoisington, D. A., Beck, D., … Frisch, M. (2004). Genetic diversity determined within and among CIMMYT maize populations of tropical, subtropical, and temperate germplasm by SSR markers. *Crop Science*, *44*, 326–334. https://doi.org/10.2135/cropsci2004.3260

SAS Institute. (2017). *SAS/STAT 14.3 user's guide*. Cary, NC: SAS Institute.

Sattler, F. T., Pucher, A., Kassari Ango, I., Sy, O., Ahmadou, I., Hash, C. T., & Haussmann, B. I. G. (2019). Identification of combining ability patterns for pearl millet hybrid breeding in West Africa. *Crop Science*, *59*, 1590–1603. https://doi.org/10.2135/cropsci2018.12.0727

Sattler, F. T., Sanogo, M. D., Kassari, I. A., Angarawai, I. I., Gwadi, K. W., Dodo, H., & Haussmann, B. I. G. (2018). Characterization of West and Central African accessions from a pearl millet reference collection for agro-morphological traits and Striga resistance. *Plant Genetic Resources: Characterization and Utilization*, *16*, 260–272. https://doi.org/10.1017/S1479262117000272

Satyavathi, C. T. (Ed). (2017). *Project coordinator review: 52nd Annual Group Meeting of the All India Coordinated Pearl Millet Improvement Project, Ludhiana, India*. Jodhpur, India: All India Coordinated Research Project on Pearl Millet. Retrieved from www.aicpmip.res.in/pcr2017.pdf

Senthilvel, S., Jayashree, B., Mahalakshmi, V., Kumar, P. S., Nakka, S., Nepolean, T., & Hash, C. T. (2008). Development and mapping of simple sequence repeat markers for pearl millet from data mining of expressed sequence tags. *BMC Plant Biology*, *8*. https://doi.org/10.1186/1471-2229-8-119

Serba, D. D., Perumal, R., Tesso, T. T., & Min, D. (2017). Status of global pearl millet breeding programs and the way forward. *Crop Science*, *57*, 2891–2905. https://doi.org/10.2135/cropsci2016.11.0936

Serba, D. D., Muleta, K. T., Amand, St. P., Bernardo, A., Bai, G., Perumal, R., & Bashir, E. (2019). Genetic diversity, population structure, and linkage disequilibrium of pearl millet. *The Plant Genome*, *12*, 1–12. https://doi.org/10.3835/plantgenome2018.11.0091

Singh, S., & Gupta, S. K. (2019). Formation of heterotic pools and understanding relationship between molecular divergence and heterosis in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *PLOS ONE*, *14*(5). https://doi.org/10.1371/journal.pone.0207463

Singh, S., Gupta, S. K., Thudi, M., Das, R. R., Vemula, A., Garg, V., … Yadav, D. V. (2018). Genetic diversity patterns and heterosis prediction based on SSRs and SNPs in hybrid parents of pearl millet. *Crop Science*, *58*, 2379–2390. https://doi.org/10.2135/cropsci2018.03.0163

Stich, B., Haussmann, B. I. G., Pasam, R., Bhosale, S., Hash, C. T., Melchinger, A. E., & Parzies, H. K. (2010). Patterns of molecular and phenotypic diversity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] from West and Central Africa and their relation to geographical and environmental parameters. *BMC Plant Biology*, *10*. https://doi.org/10.1186/1471-2229-10-216

Upadhyaya, H., Reddy, K. N., Ramachandran, S., Kumar, V., & Ahmed, M. I. (2016). Adaptation pattern and genetic potential of Indian pearl millet named landraces conserved at the ICRISAT genebank. *Indian Journal of Plant Genetic Resources*, *29*, 97–113. https://doi.org/10.5958/0976-1926.2016.00015.2

Vom Brocke, K., Christinck, A., Weltzien, R. E., Presterl, T., & Geiger, H. H. (2003). Farmers' seed systems and management practices determine pearl millet genetic diversity patterns in semi-arid regions of India. *Crop Science*, *43*, 1680–1689. https://doi.org/10.2135/cropsci2003.1680

Wang, C., Jia, G., Zhi, H., Niu, Z., Chai, Y., Li, W., … Diao, X. (2012). Genetic diversity and population structure of Chinese foxtail millet [*Setaria italica* (L.) Beauv.] landraces. *G3: Genes, Genomes, Genetics*, *2*, 769–777. https://doi.org/10.1534/g3.112.002907

Wang, K., Qiu, F., Larazo, W., Angelita, M., & Xie, F. (2015). Heterotic groups of tropical *indica* rice germplasm. *Theoretical and Applied Genetics*, *128*, 421–430. https://doi.org/10.1007/s00122-014-2441-5

Weinberg, W. (1909). Uber Vererbungsgesetze beim Menschen. *Zeitschrift fur induktive Abstammungs- und Vererbungslehre*, *1*, 377–393.

Wright, S. (1978). *Evolution and the genetics of populations: Vol. IV. Variability within and among natural populations*. Chicago, IL: University of Chicago Press.

Yadav, O. P., Bidinger, F. R., & Singh, D. V. (2009). Utility of pearl millet landraces in breeding dual-purpose hybrids for arid zone environments of India. *Euphytica*, *166*, 239–247.

Yadav, O. P., & Rai, K. N. (2013). Genetic improvement of pearl millet in India. *Agricultural Research*, *2*, 275–292. https://doi.org/10.1007/s40003-013-0089-z

Yadav, O. P., Rai, K. N., Bidinger, F. R., Gupta, S. K., Rajpurohit, B. S., & Bhatnagar, S. K. (2012). Pearl millet (*Pennisetum glaucum*) restorer lines for breeding dual-purpose hybrids adapted to arid environments. *Indian Journal of Agriculture Science*, *82*, 922–927.

Yadav, O. P., Singh, D. V., Dhillon, B. S., & Mohapatra, T. (2019). India's evergreen revolution in cereals. *Current Science*, *116*, 1805–1808. https://doi.org/10.18520/cs/v116/i11/1805-1808

Yadav, O. P., Upadhyaya, H. D., Reddy, K. N., Jukanti, A. K., Pandey, S., & Tyagi, R. K. (2017). Genetic resources of pearl millet: Status and utilization. *Indian Journal of Plant Genetic Resources*, *30*, 31–47. http://doi.org/10.5958/0976-1926.2017.00004.3

Zhang, Q., Gao, Y. J., Saghai-Maroof, M. A., Yang, S. H., & Li, J. X. (1995). Molecular divergence and hybrid performance in rice. *Molecular Breeding*, *1*, 133–142. https://doi.org/10.1007/BF01249698

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

How to cite this article: Patil KS, Gupta SK, Marathi B, et al. African and Asian origin pearl millet populations: Genetic diversity pattern and its association with yield heterosis. *Crop Science*. 2020;1–15. https://doi.org/10.1002/csc2.20245