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SPECIAL ISSUE: ADAPTING AGRICULTURE TO CLIMATE CHANGE: A WALK ON THE WILD SIDE

Use of wild *Pennisetum* species for improving biotic and abiotic stress tolerance in pearl millet

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Abstract

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is one of the world's hardiest warm-season cereal crop cultivated for food and animal feed in the semi-arid tropics of Asia and Africa. This crop faces terminal drought during rainy and flowering-stage heat stress during summer seasons. Blast is emerging as a serious threat affecting its production and productivity in India. Using wild *P. violaceum* (Lam) Rich. and pearl millet cultivars, prebreeding populations were developed following backcross method. These populations were evaluated in target ecologies in India at three locations during the 2018 summer season for flowering-stage heat stress and at two locations during the 2018 rainy season for terminal drought stress. A total 18 introgression lines (ILs) from Population (Pop) 3 exhibited improved seed set under high heat stress vs. the cultivated parent, whereas no IL was better than the cultivated parent in Pop 4. Under rainfed conditions at Hisar and Bawal, India, 19 ILs from Pop 3 and 16 ILs from Pop 4 showed significantly higher dry fodder yield than the cultivated parents. Further, screening of ILs for five diverse pathotype isolates—Pg 45, Pg 138, Pg 186, Pg 204, and Pg 232—of blast resulted in the identification of resistant ILs. Use of these promising ILs in breeding programs will assist in developing new varieties and hybrids with improved tolerance to biotic and abiotic stresses. The study indicated the genetic differences between the parents involved in crossing and also highlighted the importance of precise phenotyping of wild species for target trait prior to use in prebreeding work.

Abbreviations: BLUP, best linear unbiased predictor; G × E, genotype-by-environment; GGE-plot, genotype by G × E; IL, introgression line; Pop, population; *T*-max, maximum high temperature.

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1 | INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is an important cereal crop, after rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), and sorghum [*Sorghum bicolor* (L.) Moench], and is the major staple food crop cultivated in the drought-prone arid and semi-arid tropical regions of the world. It is an important cross-pollinated diploid ($2n = 2x = 14$) C_4 species, which is cultivated as food and fodder crop on about 31.71 million ha area with 28.36 Tg production and 894 kg ha^{-1} average productivity in more than 30 countries predominantly in Africa and Asia (Yadav & Rai, 2013). Pearl millet is primarily cultivated for grains, which are mainly used for making unleavened bread (chapatti) in southern Asia or as porridge, gruel, dumplings, couscous, and beer in Africa for human consumption, as well as for poultry feed. It is also a valuable source of fodder (both stover and green forage) and is cultivated as a forage crop in Australia, Latin America, and Brazil. It is grown as a rainfed crop with minimal inputs. Because of its ability to withstand difficult growing conditions, such as drought, low soil fertility, and high temperature, pearl millet can produce yields in areas where other cereal crops such as maize, rice, or sorghum fail to produce economic yields. Besides this, pearl millet is nutritionally better and has higher levels of iron, zinc, calcium, lipids, and proteins with more balanced amino acid profile than maize or sorghum (Rai, Hash, Singh, & Velu, 2008).

Though pearl millet is a hardy crop, its cultivation in different environments is affected by various biotic and abiotic stresses. Major biotic stresses adversely affecting pearl millet production are diseases such as downy mildew (caused by *Sclerospora graminicola*), blast (caused by *Pyricularia grisea*; teleomorph: *Magnaporthe grisea*), rust (caused by *Puccinia substriata* var. *indica*), ergot (caused by *Claviceps fusiformis*), and smut (caused by *Moesiziomyces penicillariae*). Important abiotic stresses include terminal drought (flowering through grain-filling stage), and flowering-stage heat.

India is the single largest producer of pearl millet in the world both in terms of area (6.93 million ha) and production (8.61 Tg) (Directorate of Millet Development, 2020), and the crop is mainly grown during the rainy (June–September) season with average grain yield varying from 800–1,000 kg ha^{-1} (Gupta et al., 2015). Cultivation of pearl millet in arid and semi-arid regions during the rainy season confronts challenging and hostile crop growing environment especially because of extremely low rainfall and its variable distribution throughout the growing season that exposes the crop to drought stress of variable intensity, magnitude, and duration at different growth stages. Though pearl millet is generally considered a drought-tolerant crop, terminal drought stress causes the highest yield reduction (Krishnamurthy, Zaman-Allah, Purushothaman, Irshad, & Vadez, 2011; Mahalakshmi, Bidinger, & Raju, 1987).˚

Recently, pearl millet is being extended for cultivation during the summer (February–May) season under irrigated condition in parts of Gujrat, Rajasthan, and Uttar Pradesh as the yields are better because of the high input, secured irrigation, and high plant population. In these areas, the maximum air temperatures vary from 40 to 48 ˚C and sometimes it may exceed 50 ˚C during the month of May. This high temperature usually coincides with flowering to grain-filling stage of the crop and adversely affects the crop yield potential. Though some of the commercial hybrids, which have been bred especially for such hot dry-regions, are performing reasonably well, there is a need to develop new breeding material as heavy dependence on a few cultivars might expose them to disease epidemics.

Pearl millet cultivation in arid and semi-arid tropical regions is likely to be affected by changing climatic factors, such as reduced precipitation, prolonged dry spells, intense heat waves, and possibly altered pests and diseases dynamics, and it is expected that there will be about 15% yield loss in eastern Africa by the middle of the century (Adhikari, Nejadhashemi, & Woznicki, 2015). It is reported that the ambient air temperature would rise between 1 and 3.4 ˚C by the end of this century as a result of global warming (IPCC, 2014). Overall, the crop will face more adverse climatic conditions particularly with respect to drought and heat stress. Although some genetic variations have been reported for drought and heat stress tolerance in the cultivated gene pool (Gupta et al., 2015; Yadav, 2010), this may not be sufficient to meet the challenges of increased intensity of drought and heat stress that pearl millet will be exposed to by the end of 21st century.

Crop wild relatives represent a large reservoir of genes and alleles for resistance to abiotic and biotic stresses. A few wild species such as *P. glaucum* ssp. *monodii* (Maire) Br., *P. purpureum* Schumach, and *P. squamulatum* Fresen. have been used by various researchers in past for improving cultivated pearl millet (Dujardin & Hanna, 1989; Hanna, Wells, & Burton, 1985; Jauhar & Hanna, 1998; Kannan, Valencia, & Altpeter, 2013; Kaushal et al., 2008; Obok, Aken'Ova, & Iwo, 2012). The cross-compatible wild species, *P. violaceum* (syn. *P. glaucum* ssp. *monodii* forma *violaceum* or *P. glaucum* ssp. *violaceum*) holds a great potential for pearl millet improvement. This species is found only in Sahelian region where the phenotypic diversity of pearl millet is the highest. It grows in even more arid regions having very high temperature and drought conditions than the areas where cultivated pearl millet is grown. As this species is freely crossable with cultivated pearl millet, it could be a potential source of adaptation for the cultivated type in future extreme climatic conditions. The ICRISAT genebank at Patancheru conserves 305 *P. violaceum* accessions. Screening of these accessions against five pathotypes of *Magnaporthe grisea* led to the identification

of 59 accessions having resistance against blast, an important disease of pearl millet in southern Asia and already of considerable importance in more humid areas of western Africa and in the Americas. The present study was planned to use *P. violaceum* to improve flowering-stage heat and terminal drought tolerance and blast resistance in the cultivated pearl millet.

2 | MATERIAL AND METHODS

Two *P. violaceum* accessions, IP 21544 (from Niger, Tillaberi province) and IP 21720 (Chad, Ouaddai province) having resistance to earlier reported pathotypes of blast (Sharma et al., 2013, 2020) were selected as donors, and four cultivated pearl millet genotypes including a forage germplasm line IP 22269, a forage cultivar ICMV 05555, and two hybrid parental lines, ICMB 94555 (46–47 d to flowering, d_2 dwarf type) and ICMB 97111 (48–50 d to flowering) were used as recipient parents to generate interspecific crosses in the present study. ICMB 94555 and ICMB 97111 are good general combiners, which are susceptible to different pathotypes of blast, and are seed parents of popular hybrids such as HHB 223, HHB 197 cultivated in India (Singh, Yadav, Yadav, Vart, & Yadav, 2016). Interspecific crosses were generated by using cultivated genotype as female and wild *P. violaceum* as pollen parents. A total of four interspecific crosses, IP 22269 × IP 21544, ICMV 05555 × IP 21720, ICMB 94555 × IP 21544, and ICMB 97111 × IP 21720, were generated under greenhouse conditions. In each of the four crosses, true-to-type F_1 plants were identified on the basis of morphological traits such as number of tillers per plant, stem thickness, and panicle compactness and shape. True-to-type F_1 plants were used as pollen parent and backcrossed with the respective recipient parent to generate backcross (BC_1F_1 and BC_2F_1) populations. BC_1F_1 and BC_2F_1 plants were selfed to generate four prebreeding populations (Supplemental Table S1). A large variability was observed for flowering and seed set in these prebreeding populations. In each population, introgression lines (ILs) having good seed set and early flowering were selected for further evaluation. Overall, four prebreeding populations, designated as Pop 1 (derived from IP 22269 × IP 21544 cross) consisting of 38 ILs, Pop 2 (ICMV 05555 × IP 21720 cross) with 89 ILs, Pop 3 (ICMB 94555 × IP 21544 cross) with 31 ILs, and Pop 4 (ICMB 97111 × IP 21720 cross) with 63 ILs were selected for further evaluation across locations (Supplemental Table S1).

2.1 | Field evaluation in summer season and identification of heat-tolerant ILs

Field evaluation of ILs for flowering-stage heat tolerance was carried out during the 2018 summer season across three locations, Agra in Uttar Pradesh (a site of partner Metahelix Life Sciences Ltd.), SK Nagar (a site of partner Corteva Agriscience), and Tharad (a site of partner Bayer BioSciences Pvt. Ltd.), the latter two located in Gujarat state in India. These locations were carefully selected to ensure precise phenotyping for flowering-stage heat stress (Table 1). In these locations, summer crop of pearl millet is grown from February to June with assured irrigation facility and daily maximum air temperature of ≥40 °C from boot-leaf stage (40–45 d after sowing) to seed-set stage (~15–20 d after boot-leaf stage). The screening protocol for flowering-period heat tolerance given by Gupta et al. (2015) was followed. The ILs were selected based on their adaptability in terms of flowering at Agra and SK Nagar during the 2017 summer season (data not shown). As 17 ILs of Pop I and the entire Pop 2 did not flower or flowered very late at these locations in the 2017 summer season, these ILs were not included in the evaluation study during the 2018 summer season. Overall, the screening nursery, comprised of 115 ILs (21, 31, and 63 ILs from Pop 1, Pop 3, and Pop 4, respectively) along with three cultivated recipient parents (IP 22269, ICMB 94555, and ICMB 97111), two wild donor parents (IP 21544 and IP 21720), and seven checks (ICMB 96666, 86M64, ICMB 98555, 9444, ICMB 00555, Nandi 52, and ICMB 06999), were planted at each of the three locations in the 2018 summer season. The three commercial hybrids 86M64, 9444, and Nandi 52 were used as the heat-tolerant checks, whereas two hybrid parental lines, ICMB 00555 and ICMB 06999, were used as the susceptible checks. The nursery at each location was planted two times at a 10-d interval to ensure that each IL was exposed to air temperatures of ≥40 °C during flowering period in at least one of the plantings (Gupta et al., 2015). The first planting was carried out on 10 Mar. 2018 at Agra and on 28 Feb. 2018 at SK Nagar and Tharad; the second planting was carried out on 20 Mar. 2018 at Agra, on 9 Mar. 2018 at SK Nagar, and on 10 Mar. 2018 at Tharad. The plot size was a two-row plot of 4-m row length for each entry in single replication.

Data loggers (U23-001, HOBO Pro v2 Temp/RH) were installed in the experimental fields at an average panicle height (0.91–1.5 m above ground) at all the three locations. Data loggers were programmed to record air temperature and relative humidity every half hour. Data on all the weather parameters such as maximum high temperature (*T*-max) at boot-leaf stage during day and night as well as relative humidity at *T*-max at boot-leaf stage were recorded at Agra, SK Nagar and Tharad. Normal package of practices was followed to raise good crop and the nursery was irrigated at regular intervals to avoid drought stress. In each trial, five random plants in each entry were tagged at boot-leaf stage at each location. Data were recorded on days to boot-leaf stage, days to 50% flowering, and seed set in percentage (at physiological maturity) in each planting across all three locations. The data from a particular site was considered only when susceptible

TABLE 1 Coordinates and temperatures of testing locations during boot-leaf stage of pearl millet prebreeding materials during summer season of 2018 at Agra, SK Nagar and Tharad, India

Location	Latitude	Longitude	Elevation	Temperature			
				First planting		Second planting	
				Max.[a]	Min.[b]	Max.[a]	Min.[b]
	˚N	˚E	m asl	˚C			
Agra	27.17	78.00	166	29.6–51.4 (32)	23.1–34.1 (21)	33.7–51.4 (31)	23.6–31 (24)
SK Nagar	24.30	72.13	152.5	35.9–43.6 (27)	15.3–33.6 (10)	37.9–45.4 (21)	15.6–24.9 (0)
Tharad	24.03	71.41	10	38.2–44.3 (20)	14.6–23.9 (0)	38.1–44.3 (24)	14.6–23.9 (0)

[a]Number in parenthesis indicates no. of days when max. day temperature exceeded 40 ˚C at a given location.
[b]Number in parenthesis indicates no. of days when min. night temperature exceeded 26 ˚C at a given location.

checks had seed set of <50% and resistant checks had seed set of >70%. The seed set data of only those plants in a particular entry were considered for analysis that were exposed to *T*-max of ≥40 ˚C at that particular site (as per data logger installed in the experiment).

2.1.1 | Statistical analysis

In the 2018 summer season, all the ILs and control cultivars encountered air temperatures of ≥40 ˚C at least at one or both the planting dates at one or more locations. Because of photoperiod sensitivity in IP 22269, which led to the late flowering in 21 ILs of Pop 1 derived from IP 22269, these genotypes were excluded from screening. In Pop 3 and Pop 4, the seed set data of only those plants of a particular entry were considered for analysis where days to boot-leaf stage coincided with *T*-max of ≥40 ˚C. The seed set data for all such plants of a particular entry were averaged across two plantings at each location to find mean seed set in percentage. The mean seed set of an entry was averaged over all the three locations to find overall mean seed set (%). The overall mean seed set (%) of ILs was compared with the respective cultivated parent to identify the useful recombinants, and the ILs were classified into different seed set classes. The ILs having ≥70% overall mean seed set were considered as the heat tolerant ILs.

2.2 | Field evaluation in rainfed condition and identification of promising ILs

The field evaluation for terminal drought tolerance was carried out at two locations (Bawal and Hisar) of Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar, India, during the 2018 rainy seasons; these two locations are in drought-prone ecology, which falls under rainfall zone of <400 mm yr^{-1}. A nursery comprised of 115 ILs (21 ILs from Pop 1, 31 ILs from Pop 3, and 63 ILs from Pop 4), three recipient parents (IP 22269, ICMB 94555, and ICMB 97111), along with 10 checks (six drought tolerant checks having early maturity [Hybrids: HHB 67 Improved, RHB 177, RHB 173, MPMH 17, GHB 538, GHB 719]; and three drought tolerant checks having late maturity [Hybrids 9444, 86M86, and 'Kaveri Super Boss'], along with a hybrid parental line: ICMB 04999) were planted at Hisar and Bawal.

Amongst the checks, HHB 67 Improved, RHB 177, RHB 173, MPMH 17, GHB 538, and GHB 719 are the early maturing drought-tolerant commercial hybrids; ICMB 04999 is an early maturing B-lines of the commercial drought tolerant hybrids RHB 223, MPMH 17, and GHB 905; and 9444, 86M86, and Kaveri Super Boss are the late-maturing hybrids. At each location, the nursery was planted in two replications and each genotype was planted in a two-row plot of 4-m row length. Data were recorded on days to 50% flowering, plant height (cm), panicle weight (kg ha^{-1}), grain yield (kg ha^{-1}), and dry fodder yield (kg ha^{-1}). Panicle harvest index was calculated as grain yield (kg ha^{-1}) / panicle weight (kg ha^{-1}) and expressed as a percentage (%).

2.2.1 | Statistical analysis

Individual and combined analysis of variance was performed on phenotypic data recorded during 2018 rainy season to test the significance of genotype (G), environment (E) and genotype × environment (G × E) interaction using MIXED procedure in SAS 9.4 (SAS Institute Inc, 2018) considering replication as fixed and environment and genotypes as random effects. Individual location variances were estimated and modeled into combined analysis with REPEATED statement. Variance components were determined as genotypes (σ^2_g), genotype × environment interaction (σ^2_{gxe}), and error (σ^2_e). Best linear unbiased predictors (BLUPs) were estimated for G, E, and G × E, and multiple comparisons were performed for significant effects using *t*-test. Based on BLUPs, the range, mean, variances, and broad-sense heritability (H^2) were computed. The performance of ILs was compared with the respective cultivated parent for days to 50% flowering and dry fodder yield at each location as well as pooled and the agronomically superior ILs were identified for these traits. The

environment-centered genotype by G × E (GGE-plot) was plotted using Hisar location on *Y*-axis and Bawal on *X*-axis to identify location specific superior genotypes for dry fodder yield. All the entries were given unique GGE-plot numbers. The line $Y = X$ was plotted wherein the ILs above the $Y = X$ line performed better at Hisar in the 2018 rainy and ILs below the $Y = X$ line performed better at Bawal in the 2018 rainy season.

2.3 | Evaluation for blast resistance

Screening of prebreeding populations against five pathotype isolates—Pg 45, Pg 138, Pg 186, Pg 204, and Pg 232—of *M. grisea* was carried out under controlled environmental conditions at ICRISAT, Patancheru. The four prebreeding populations consisting of 221 ILs (38, 89, 31, and 63 ILs from Pop 1, Pop 2, Pop 3, and Pop 4, respectively) were screened against five pathotype isolates along with six parents (four recipients and two donors) and checks ICMB 95444 (highly blast susceptible line) and IP 21187-P1 (resistant to the isolates used for screening except Pg 138). The cultures *M. grisea* isolates used in this study were obtained from the culture collection being maintained in the Cereals Pathology Lab, ICRISAT, Patancheru. Multiplication of test isolates for the screening of ILs was carried out by subculturing on oatmeal agar medium and incubating at 25 ± 1 ˚C with 12 h of darkness for 7–10 d. The spores of each isolate were harvested in sterile distilled water by following the procedure described by Sharma et al. (2013). The spore suspension was filtered through sterilized muslin cloth and the spore concentration was adjusted to 1×10^5 spores ml^{-1} by using a haemocytometer. For the uniform dispersal of spores, two to three drops of Tween 20 were added to the suspension just before inoculation.

Seeds of 221 ILs, parents, and the checks were sown in 10-cm diam. pots filled with sterilized soil–sand–farmyard manure mix (2:1:1 by volume). The pots were placed in a greenhouse bay maintained at 30 ± 2 ˚C for 14 d for the germination of seed and growth of seedlings. The 12-d-old seedlings were inoculated with the spore suspension of each isolate separately using atomizer sprayer, the pots were covered with polyethylene bags immediately after inoculation, and incubated at 25 ± 1 ˚C for 24 h. The polyethylene bags were removed after 24 h of incubation, the pots were transferred to greenhouse benches, and the inoculated seedlings were exposed to >90% relative humidity under misting for 6 d. The blast screening experiments were conducted separately against each isolate in completely randomized design with two replicates: one pot per replicate with 12–15 seedlings. Poor germination was observed in some ILs; hence, the number of seedlings screened for these ILs was quite low. The blast severity was recorded on each seedling against each isolate using a 1-to-9 progressive rating scale after 8 d of inoculation (Sharma et al., 2013). The plants that scored as 3 or less were considered resistant.

In most of the ILs, both resistant and susceptible plants were observed within individual ILs against test isolates. Hence, resistant seedlings (maximum of three to four resistant plants per IL) were selected, transplanted in 25-cm pots, placed in open space outside the greenhouse, and grown until maturity. The heads of the selected and transplanted plants were covered using selfing bags upon emergence from the flag leaf to produce selfed seed. This first screening cycle from which resistant plants were selected from each IL was considered as S_0. The selfed seeds were rescreened in the next two screening cycles, S_1 and S_2, to stabilize resistance following selfing and rescreening. The seedlings raised from selfed seed of resistant plants from S_0 were screened against specific pathotype isolates in the S_1 screening cycle. Similarly, seedlings raised from selfed seed of resistant plants selected from the S_1 screening were further screened for blast resistance in the S_2 screening cycle. As a whole, the progenies of single-selected resistant plant per IL were screened each in the S_1 and S_2 screening cycles against Pg 45, Pg 138, Pg 186, Pg 204, and Pg 232 to identify stable pathotype-specific blast-resistant ILs derived from four populations.

2.3.1 | Statistical analysis

Analysis of variance (ANOVA) was carried out for mean blast scores of ILs derived from four populations against five pathotype isolates separately using the GENSTAT statistical package (version 10.1; Rothamsted Experiment Station, Herpenden, Herts, UK) to determine significant differences among ILs and populations for blast reaction (Payne, Murray, Harding, Baird, & Soutar, 2009).

3 | RESULTS

3.1 | Flowering-stage heat tolerance

In the 2018 summer season, the *T*-max at the boot-leaf stage in the first and second planting at Agra varied from 29.6–51.4 ˚C. A total 32 d exceeded the *T*-max of >40 ˚C in the first planting and 21 d in the second planting (Table 1). At SK Nagar, the *T*-max at boot-leaf stage varied from 35.9 to 45.4 ˚C, wherein 27 d exceeded the *T*-max of >40 ˚C in the first planting and 21 d in the second planting. At Tharad, the *T*-max at boot-leaf stage varied from 38.2 to 44.3 ˚C with 20 d in the first planting and 24 d in the second planting exceeding the *T*-max of >40 ˚C. The relative humidity at the respective *T*-max at both the planting varied from 7.9 to 61.4% at Agra, from 10.9 to 41% at SK Nagar, and from 15.5 to

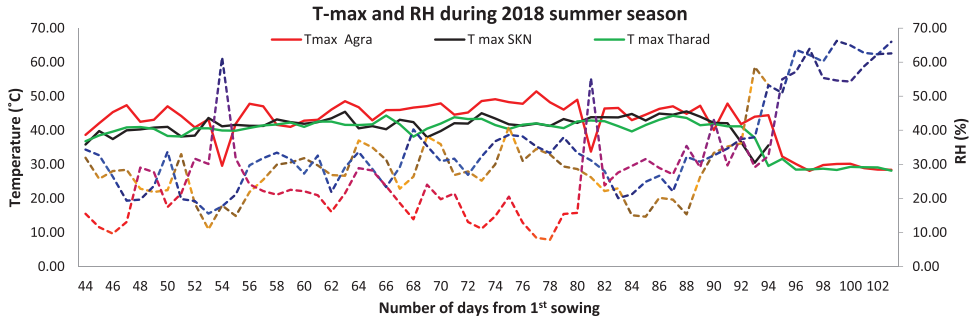

FIGURE 1 Daily maximum air temperature (*T*-max) and relative humidity (RH) at *T*-max, during boot-leaf stage (BLS) in 2018 summer season at Agra, SK Nagar, and Tharad, India

TABLE 2 Grouping of introgression lines (ILs) based on the overall mean seed set into different seed set classes under maximum air temperature (*T*-max) of ≥40 ˚C during 2018 summer season across three locations in India

Population	No. of ILs	0–20%	20–40%	40–60%	60–69%	≥70%	No of superior ILs
ICMB 94555 (cultivated parent of Pop 3)	–	–	–	41%	–	–	–
Pop 3	23	0 (0%)[a]	5 (22%)	18 (78%)	0 (0%)	0 (0%)	18 (44–59%)
ICMB 97111 (cultivated parent of Pop 4)	–	–	–	–	–	84%	–
Pop 4	39	0 (0%)	3 (8%)	17 (43%)	10 (26%)	9 (23%)	None
Overall populations	62	0 (0%)	8 (13%)	35 (56%)	10 (16%)	9 (15%)	

[a]Values in parenthesis indicate percentage introgression lines within that population.

40.3% at Tharad. The daily *T*-max and relative humidity at Agra, SK Nagar, and Tharad during the crop season are given in Figure 1.

Based on the criterion for selecting ILs where the boot-leaf stage coincided with the *T*-max of ≥40 ˚C, a total of 71 genotypes (62 ILs, two recipient parents, and seven checks) were selected for further analysis. As both the wild donor parents (IP 21544 and IP 21720) from Africa did not flower at any of these locations in India as a result of photoperiod sensitivity, the heat tolerance levels of these accessions could not be determined. The boot-leaf stage in the selected 71 genotypes varied from 47 to 62 d with an average of 52 d, and the overall mean seed set ranged from 30% (ICMB 00555 and PBPMPOP 3−101) to 91% (86M64) across three locations. In selected 62 ILs, the overall mean seed set varied from 30% (PBPMPOP 3–101) to 75% (PBPMPOP 4–175) with an average of 55% seed set. Cultivated parents ICMB 94555 and ICMB 97111 of Pop 3 and Pop 4 had 41 and 84% seed set, respectively. Amongst checks, the overall mean seed set varied from 87 (Nandi 52) to 91% (86M64) with 89% mean seed set in heat tolerant hybrids, and from 70 (ICMB 98555) to 76% (ICMB 96666) with 73% mean seed set in heat-tolerant hybrid parental lines. The heat-susceptible checks recorded 30 (ICMB 00555) to 50% (ICMB 06999) overall mean seed set.

A large variability was observed at individual location as well. At Agra, the boot-leaf stage in these selected genotypes varied from 48 to 67 d with an average of 54 d and the mean seed set among ILs, and checks varied from 14 (PBPMPOP 3–101) to 94% (86M64) with an average of 59% seed set. Similarly, at SK Nagar, the boot-leaf stage varied from 46 to 63 d (mean 54 d) and the mean seed set from 17 (ICMB 94555) to 95% (Nandi 52) with an average of 71% seed set. The boot-leaf stage varied from 44 to 58 d (mean 49 d) and the mean seed set ranged from 10 (PBPMPOP 3–108) to 92% (86M64) at Tharad (40% mean seed set).

Based on the overall mean seed set (%) across locations, 62 ILs were grouped into four different classes (Table 2). The majority of the ILs showed 40–60% seed set. In Pop 3, 18 ILs had higher seed set (44–59%) than the cultivated parent ICMB 94555 (41%). In Pop 4, none of the ILs had seed set better than the cultivated parent. However, nine ILs, namely PBPMPOP 4–157 (72%), PBPMPOP 4–160 (70%), PBPMPOP 4–168 (72%), PBPMPOP 4–170 (72%), PBPMPOP 4–174 (73%), PBPMPOP 4–175 (75%), PBPMPOP 4–176 (74%), PBPM-

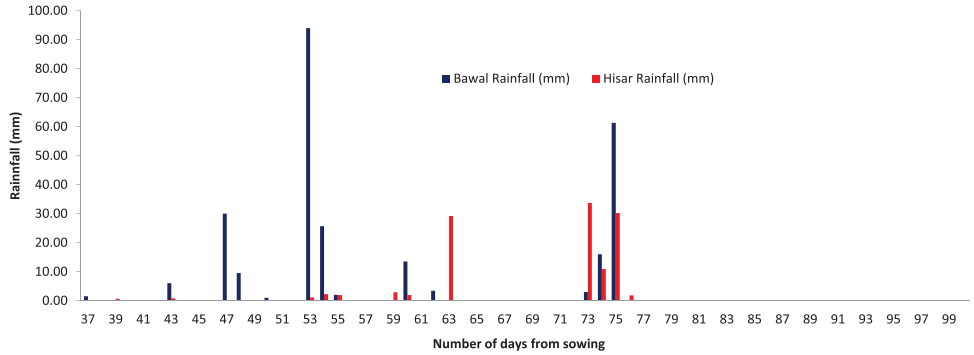

FIGURE 2 Daily rainfall during 2018 rainy season at Hisar and Bawal, India

POP 4–178 (72%), and PBPMPOP 4–195 (72%), exhibited ≥70% overall mean seed set across three locations, which is desirable in pearl millet breeding programs (Tables 2 and 3).

3.2 | Performance of ILs in drought-prone ecology

The rainfall recorded during the 2018 crop season at Hisar and Bawal was 258.7 and 368.0 mm, respectively. There was ~39 mm rainfall at Hisar and ~80 mm rainfall at Bawal after onset of flowering to grain-filling stage (Figure 2). The objective of this study was to evaluate performance of ILs under terminal drought stress. The rainfall pattern after flowering at both Hisar and Bawal were favorable for plant growth and seed development. Hence, the true potential of these ILs under terminal drought could not be assessed. Subsequently, the testing of these ILs under controlled terminal drought conditions is suggested to confirm the presence of drought-tolerance alleles in these newly developed ILs. Data generated in the present study was therefore investigated for the variability among ILs for grain and forage yield and their dependent traits under rainfed ecology compared with the cultivated parents and checks. The restricted maximum likelihood analysis showed significant variations among ILs (σ^2_g) for all the six agronomic traits, days to 50% flowering, plant height, panicle weight, grain yield, dry fodder yield, and panicle harvest index (%) at Bawal and Hisar. Pooled analysis also showed significant genetic variance (σ^2_g) and significant G × E interactions for all agronomic traits (Table 3).

A large variation was observed amongst the ILs compared with cultivated parents and checks for all traits at each location and in pooled across two locations (Table 4). Overall, a flowering window of 39 d (varying from 49–88 d) was observed in ILs across locations that shows the presence of substantial population variability. Three cultivated parents took 46 (ICMB 97111) to 85 d (IP 22269) to 50% flowering. All early checks flowered in ~46 d (42–53 d) amongst which the popular hybrid,HHB 67 Improved took 42 d and the hybrid parental line ICMB 04999 took 53 d for 50% flowering (Table 4). Similarly, large variations were observed amongst ILs for other traits such as plant height, ranging from 128 to 207 cm (average 168 cm); panicle weight, ranging from 286 to 3,218 kg ha^{-1} (average 2,001 kg ha^{-1}); grain yield, ranging from 46 to 1,856 kg ha^{-1}; dry fodder yield, ranging from 4,375 to 11,273 kg ha^{-1}; and panicle harvest index (%), ranging from 11.5 to 68.3% (average 48.7%) (Table 4). A similar pattern for variability was observed at individual locations (Table 4). The broad-sense heritability was high for all the traits at Bawal and medium to high at Hisar (Table 4).

In order to identify the promising recombinants, the performance of ILs was compared with the respective cultivated parent for important traits such as days to 50% flowering and dry fodder yield in each location and pooled analysis (Table 4). In pooled analysis, 17 ILs in Pop 1, one IL in Pop 3, and one IL in Pop 4 flowered earlier than the respective cultivated parents. Dry fodder yield of 31 ILs in Pop 3 and 62 ILs in Pop 4 was better than the respective cultivated parents (Table 4).

Based on the environment-centered GGE plot, ILs performing better at specific locations are depicted in Supplemental Figure S1 for dry fodder yield. Among ILs, PBPMPOP 1–10, PBPMPOP 1–41, PBPMPOP 1–34, PBPMPOP 1–38, and PBPMPOP 1–24 performed better at Hisar, whereas PBPMPOP 1–4, PBPMPOP 1–23, PBPMPOP 3−103, PBPMPOP 3−87, and PBPMPOP 3−93 performed better at Bawal for dry fodder yield (Supplemental Table S2).

3.3 | Blast resistance

There was significant variation in the mean blast scores of ILs and populations as a whole against each pathotype, indicating differences in the resistance level of ILs derived from different populations (Table 5). Blast score in the susceptible check ICMB 95444 was quite high. Mean blast scores of parents and the checks are given in the Supplemental Table S3. All the seedlings of ICMB 94555 and ICMB 97111, recipient parents of Pop 3 and Pop 4 exhibited compatible disease

TABLE 3 Variance components resulting from genotypes (σ^2_g), genotype × environment ($\sigma^2_{g\times e}$) and standard errors (SE) for different traits in pearl millet prebreeding populations evaluated at Hisar and Bawal and pooled across locations during 2018 rainy season

Traits	2018 rainy season				Pooled			
	Hisar		Bawal					
	σ^2_g	SE	σ^2_g	SE	σ^2_g	SE	$\sigma^2_{g\times e}$	SE
Days to 50% flowering (d)	59.09**	7.55	69.21**	8.88	60.53**	7.94	4.08**	0.66
Plant height (cm)	383.09**	69.35	371.01**	53.68	257.64**	54.65	129.49**	32.73
Panicle weight (kg ha^{-1})	1,397,423**	197,959	430,767**	60,954	812,729**	126,591	68,025**	27,830
Grain yield (kg ha^{-1})	902,447**	126,754	131,451**	19,414	371,907**	69,642	107,967**	25,836
Dry fodder yield (kg ha^{-1})	2,870,591**	528,340	3,370,468**	475,020	1,870,769**	400,848	1,318,144**	281,140
Panicle harvest index (%)	173.89**	24.38	102.52**	17.62	99.51**	27.55	63.18**	15.03

**Significant at .01 probability level.

reaction to all the test isolates, whereas seedling of other parents recorded mixed (both resistant and susceptible) reaction. None of the ILs was completely resistant to Pg 138, whereas some lines, especially from Pop 2, exhibited high level of resistance to other pathotype isolates. However, both resistant and susceptible plants were observed in many ILs against test isolates; minimum resistant plants were observed against Pg 138. Resistant plant selections were made in 31 ILs from Pop 1, 88 from Pop 2, eight from Pop 3, and 30 from Pop 4 against Pg 45. Similarly, a number of single-plant selections against Pg 138 were six from Pop 1, 13 from Pop 2, and three from Pop 3, and no selection was made from Pop 4. Maximum single-plant selections were made against Pg 186: 36 selections in Pop 1, 88 selections in Pop 2, 22 selections in Pop 3, and 51 selections in Pop 4. Against Pg 204, 34, 88, 7, and 31 selections were made from Pop 1, 2, 3, and 4, respectively. Number of selections were minimum against Pg 232: six from Pop 4 and 78 from Pop 2.

There was an increase in the percentage of resistant plants in S_1 generation when selfed seed of selected resistant plant (S_0 screening cycle) were screened against different pathotype isolates. Further, increase in occurrence of resistant plants was also observed in the S_2 screening cycle compared with S_1 in some population–pathotype combinations, whereas slight decrease in percentage resistant plants was also observed in some lines (Figure 3). Pathotype-specific stable single-plant selections of ILs were identified showing 100% resistant plants in S_1 and S_2 screening cycles following initial selection from segregating S_0 (Supplemental Table S3). Sixty-one ILs (two in Pop 1, 44 in Pop 2, one in Pop 3, and 14 in Pop 4) were stabilized for resistance against Pg 45 after three generations of single-plant selection and screening. Similarly, 41 ILs (nine in Pop 1, 18 in Pop 2, and 14 in Pop 4) were stabilized for blast resistance against Pg 186, 66 ILs (nine in Pop 1, 42 in Pop 2, two in Pop 3, and 13 in Pop 4) against Pg 204, and 20 ILs (19 in Pop 2, and one in Pop 4) against Pg 232 (Supplemental Table S3). Some promising lines with stable resistance (100% resistant plants) in all the three screening cycles, S_0, S_1, and S_2, were also observed. Eleven ILs exhibited stable resistance in S_0, S_1, and S_2 against Pg 45, nine against Pg 186, 12 against Pg 204, and only two against Pg 232. However, none of the ILs showed 100% resistant plants in all the three screening cycles against Pg 138. Resistant plants were also selected against different pathotypes from the same ILs. The single-plant selections from some of these ILs were found to have resistance to two or more pathotype isolates, maximum being in Pop 2, indicating multiple pathotype resistance in some of the ILs. Selections from the ILs PBPMPOP 1–24 and PBPMPOP 4–153 were resistant to Pg 45, Pg 186, and Pg 204. Similarly, selections from 11 ILs from Pop 2 were resistant to any three of the five pathotype isolates used in the present study (Table 6). Stable resistant lines to any two pathotype isolates were selected from two ILs of Pop 1 and 10 ILs of Pop 4.

TABLE 4 Summary statistics for various traits in pearl millet prebreeding populations evaluated at Hisar, Bawal, and pooled across locations during 2018 rainy season

Environment	Component	Days to 50% flowering	Plant height	Panicle weight	Grain yield	Dry fodder yield	Panicle harvest index (PHI)
		d	cm	kg ha^{-1}			%
2018 rainy, Hisar	Mean (ILs)[a]	51	191	2929	1763	8541	56.1
	Range (ILs)	44–85	154–225	985–4.494	270–2,814	6,281–13,019	9.2–68.4
	Heritability	98	68	86	88	66	79
	Early tolerant group	42 (37–46)	191 (121–214)	5,222 (2,671–6,301)	3,624 (1,721–4,703)	10,114 (6,434–12,138)	68.3 (64.8–73.3)
	HHB 67 Improved	37	195	5,359	3,583	10,998	66.6
	ICMB 04999	46	121	2,671	1,721	6,434	64.8
	Cultivated parents						
	IP 22269 (cultivated parent of Pop 1)	69	270	1,211	381	11,198	22.9
	Pop 1 (range)	49–85	174–225	985–3,186	270–1,765	8,375–13,019	9.2–58.4
	Agronomically superior ILs	17 ILs early: 49–68	21 ILs with short plant height: 174–225	20 ILs with high panicle weight: 1,246–3,186	19 ILs with high grain yield: 434–1,765	Two ILs with high dry fodder yield: 11,744–13,019	18 ILs with high PHI: 25.4-58.4
	ICMB 94555 (cultivated parent of Pop 3)	47	NA	2,234	1,288	7,055	58.8
	Pop 3 (range)	44-56	154–211	1,722–4,494	730–2,814	6,900–10,378	40.5–65.7
	Agronomically superior ILs	One IL early: 44 days	26 ILs with short plant height: 154–205	28 ILs with high panicle weight: 2,258–4,494	23 ILs with high grain yield: 1,373–2,814	30 ILs with high dry fodder yield: 7,328–10,378	13 ILs with high PHI: 59.3-65.7
	ICMB 97111 (cultivated parent of Pop 4)	46	173	3,899	2,355	7,283	59.3
	Pop 4 (range)	45–51	169–208	2,739–4,137	1,671–2,806	6,281–9,586	52.9–68.4
	Agronomically superior ILs	Nine ILs early: 45 days	59 ILs taller than cultivated parent: 174–208	One IL with high panicle weight: 4,137	18 ILs with high grain yield: 2366–2,806	57 ILs with high dry fodder yield: 7,374–9,586	54 ILs with high PHI: 59.7–68.4

(Continues)

TABLE 4 (Continued)

Environment	Component	Days to 50% flowering	Plant height	Panicle weight	Grain yield	Dry fodder yield	Panicle harvest index (PHI)
2018 rainy, Bawal	Mean (ILs)	63	145	1,259	558	4,006	44.2
	Range (ILs)	54–89	91–193	537–2,355	179–1,151	1,034–9,545	21.4–66.7
	Heritability	98	93	85	90	90	91
	Early tolerant group	50 (47–59)	144 (87–165)	2,650 (1,633–3,184)	1,383 (744–1,714)	4,355 (2,210–7,260)	52.0 (46.7–55.2)
	HHB 67 Improved	47	148	2,406	1,327	3,441	55.2
	ICMB 04999	59	87	1,633	744	2,210	46.7
	Cultivated parents						
	IP 22269 (cultivated parent of Pop 1)	85	–	–	–	15,432	–
	Pop 1 (range)	63–89	–	–	–	1,034–9,545	–
	Agronomically superior ILs	16 ILs early: 63–84 days	–	–	–	–	
	ICMB 94555 (cultivated parent of Pop 3)	60	112	1,231	702	2,486	55.8
	Pop 3 (range)	56–67	91–193	570–1,919	179–877	2,061–7,169	21.4–66.7
	Agronomically superior ILs	One IL early: 56 days	26 ILs taller than cultivated parent: 114–193	11 ILs with high panicle weight: 1,284–1,919	Five ILs with high grain yield: 715–877	30 ILs with high dry fodder yield: 2,511–7,169	Four ILs with high PHI: 58.8–66.7
	ICMB 97111 (cultivated parent of Pop 4)	57	138	1,192	737	2,023	59.9
	Pop 4 (range)	54–67	115–175	769–2,355	300–1,151	1,898–5,003	26.4–66.2
	Agronomically superior ILs	Five ILs early: 54–56 days	49 ILs taller than cultivated parent: 139–175	37 ILs with high panicle weight: 1,205–2,355	13 ILs with high grain yield: 740–1,151	61 ILs with high dry fodder yield: 2,411–5,003	Three ILs with high PHI: 62.3–66.2

(Continues)

TABLE 4 (Continued)

Environment	Component	Days to 50% flowering	Plant height	Panicle weight	Grain yield	Dry fodder yield	Panicle harvest index (PHI)
Pooled	Mean (ILs)	57	168	2,001	1,127	6,273	48.7
	Range (ILs)	49–88	128–207	286–3,218	46–1,856	4,375–11,273	11.5–68.3
	Heritability	96	68	88	80	62	71
	Early tolerant group	46 (42–53)	167 (102–187)	383 (2,345–4,440)	2,437 (1,281–3,040)	7,236 (4,219–9,955)	60.4 (55.9–64.6)
	HHB 67 Improved	42	171	3,703	2,391	7,156	61.2
	ICMB 04999	53	102	2,345	1,281	4,219	55.9
	Cultivated parents						
	IP 22269 (cultivated parent of Pop 1)	77	234	482	190	14,417	22.4
	Pop 1 (range)	57–88	154–205	286–2,713	46–1,337	4,445–11,273	19.7–50.9
	Agronomically superior ILs	17 ILs early: 57–76 days	21 ILs with short plant height: 154–205	20 ILs with high panicle weight: 513–2,713	19 ILs with high grain yield: 46–1,337	–	18 ILs with high PHI: 24.5–50.9
	ICMB 94555 (cultivated parent of Pop 3)	53	167	1,982	1,126	4,614	57.8
	Pop 3 (range)	50–61	128–207	1,442–3,005	564–1,783	4,740–8,229	29.1–60.5
	Agronomically superior ILs	One IL early: 50	19 ILs taller than cultivated parent: 169–207	15 ILs with high panicle weight: 2,086–3,005	13 ILs with high grain yield: 1,150–1,783	31 ILs with high dry fodder yield: 4,740–8,229	Two ILs with high PHI: 59.6–60.5
	ICMB 97111 (cultivated parent of Pop 4)	51	155	2,349	1,524	4,450	60.2
	Pop 4 (range)	49–57	149–188	1,764–3,218	1,056–1,856	4,375–7240	42.4–68.3
	Agronomically superior ILs	One IL early: 49 days	58 ILs taller than cultivated parent: 157–188	26 ILs with high panicle weight: 2,373–3,218	11 ILs with high grain yield: 1,530–1,856	62 ILs with high dry fodder yield: 4,500–7,240	Five ILs with high PHI: 60.6–68.3

[a]IL, introgression line.

TABLE 5 Analysis of variance for blast severity in introgression lines derived from four crosses or populations against five pathotype isolates Pg 45, Pg 138, Pg 186, Pg 204, and Pg 232 of *Magnaporthe grisea* under greenhouse screening at ICRISAT, Patancheru, during 2018

Source of variation	Degree of freedom	Sum of squares	Mean square	*F*-ratio	*F*-probability
Pg 45					
Replication	1	0.0698	0.0698	0.1	–
Introgression line	220	1,012.3392	4.6015	30.08	<.001
Population or cross	3	738.6788	246.2263	348.34	<.001
Pg 138					
Replication	1	0.0052	0.0052	0.03	–
Introgression line	220	153.0305	0.6956	3.44	<.001
Population or cross	3	56.7833	18.9278	58.76	<.001
Pg 186					
Replication	1	0.0358	0.0358	0.25	–
Introgression line	219[a]	627.6702	2.8661	20.34	<.001
Population or cross	3	416.4556	138.8185	248.88	<.001
Pg 204					
Replication	1	0.0469	0.0469	0.31	–
Introgression line	220	941.302	4.2786	28.67	<.001
Population or cross	3	751.9014	250.6338	491.29	<.001
Pg 232					
Replication	1	0.5043	0.5043	1.68	–
Introgression line	220	1,186.144	5.3916	17.92	<.001
Population or cross	3	907.94	302.6467	382.07	<.001

[a]No germination in one line.

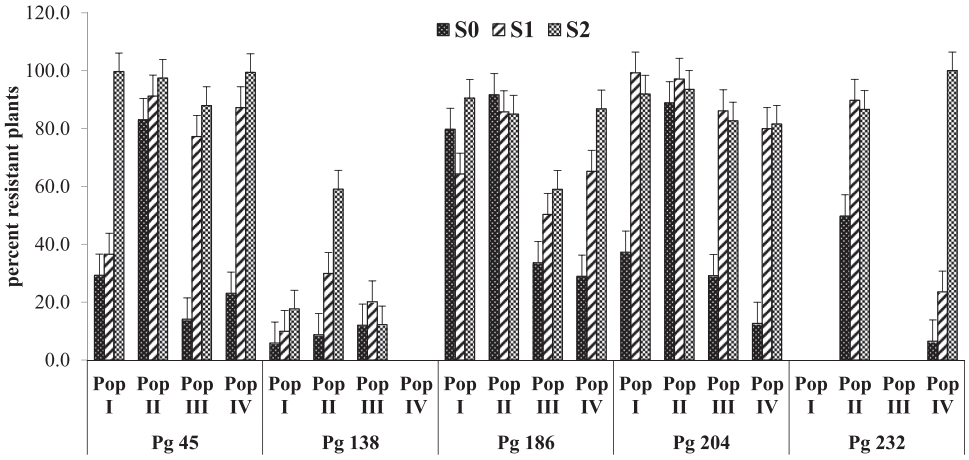

FIGURE 3 Percentage resistant plants in S0, S1, and S2 screening cycles of introgression lines derived from four populations against pathotype isolates Pg 45, Pg 138, Pg 186, Pg 204, and Pg 232

4 | DISCUSSION

The present study was focused on utilizing the genetic variability present in *P. violaceum* for blast resistance and drought and heat tolerance. This species is found only in the Sahelian region where it grows in even more arid regions than the cultigen and could be a potential source of adaptation for the cultivated pearl millet in the future extreme climatic conditions. Two accessions of *P. violaceum*, IP 21544 and IP 21720, originating from Niger and Chad, respectively were selected from the blast resistant accessions identified from the screening of 305 *P. violaceum* accessions against *M. grisea*

TABLE 6 Promising introgression lines (ILs) with multiple pathotype-resistant single-plant selections (× = resistant to pathotype)

	Resistant to *M. grisea* pathotype			
Selection from IL	**Pg 45**	**Pg 186**	**Pg 204**	**Pg 232**
PBPMPOP 1–24	×	×	×	–
PBPMPOP 1–30	×	×	–	–
PBPMPOP 1–7	–	×	×	–
PBPMPOP 4–122	–	×	×	–
PBPMPOP 4–153	×	×	×	–
PBPMPOP 4–118	×	–	×	–
PBPMPOP 4–120	×	–	×	–
PBPMPOP 4–155	×	–	–	×
PBPMPOP 4–165	×	×	–	–
PBPMPOP 4–171	×	×	–	–
PBPMPOP 4–190	×	–	×	–
PBPMPOP 4–196	×	×	–	–
PBPMPOP 4–122	–	×	×	–
PBPMPOP 4–192	–	×	×	–
PBPMPOP 2–211	×	×	×	–
PBPMPOP 2–48	×	×	×	–
PBPMPOP 2–53	×	×	×	–
PBPMPOP 2–55	×	×	×	–
PBPMPOP 2–68	×	–	×	×
PBPMPOP 2–69	×	–	×	×
PBPMPOP 2–70	×	×	×	–
PBPMPOP 2–71	×	×	×	–
PBPMPOP 2–72	×	–	×	×
PBPMPOP 2–73	×	–	×	×
PBPMPOP 2–76	×	–	×	×

pathotype isolates Pg 45, Pg 53, Pg 56, Pg 118, and Pg 119 (Sharma, Sharma, & Gate, 2020). These two *P. violaceum* accessions along with four cultivated pearl millet genotypes, IP 22269, ICMV 05555, ICMB 94555, and ICMB 97111, were used to generate four prebreeding populations, Pop 1 (derived from IP 22269 × IP 21544 cross), Pop 2 (ICMV 05555 × IP 21720 cross), Pop 3 (ICMB 94555 × IP 21544 cross), and Pop 4 (ICMB 97111 × IP 21720 cross) following backcross breeding.

With an objective to identify flowering-stage heat-tolerant ILs, the prebreeding populations (Pop. 3 and 4) were evaluated across three locations: Agra, SK Nagar, and Tharad during the 2018 summer season. Though pearl millet is relatively heat tolerant than other major cereal crops, very high temperature (≥40 ˚C) at reproductive stages (boot-leaf stage and stigma emergence–anthesis stage) shows detrimental effects that may have severe consequences on the seed set and grain yield as a whole (Gupta et al., 2015). Under very high temperature, of up to 51.4 ˚C at Agra, 45.4 ˚C at SK Nagar, and 44.3 ˚C at Tharad, the ILs showed a large variability for seed set at each of the three locations. Overall, a maximum of 32 d exceeded the *T*-max of >40 ˚C at boot-leaf stage at Agra, 27 d at SK Nagar, and 24 d at Tharad. This indicates that there was continuous imposition of heat stress on the test entries during flowering stage. A large variability was observed among the test entries for seed set. Change in seed set under heat stress has been reported as a result of the genotypic differences in pearl millet (Gupta et al., 2015; Prasad, Bheemanahalli, & Jagadish, 2017) and maize (Basetti & Westgate, 1993; Otegui & Melón, 1997). Pop 3 was derived from ICMB 94555 (41% seed set) and the wild donor parent, IP 21544, and Pop 4 was derived from ICMB 97111 (84% seed set), and the wild donor parent, IP 21720. These cultivated parental lines were involved in crosses, owing to their better combining ability, and their flowering-period heat stress response was not known. As the wild accessions grow in more arid regions of Niger, it was speculated that these accessions might have high heat and drought tolerance compared with the cultivated pearl millet. In Pop 3, seed-set percentage in 18 ILs (PBPMPOP 3–78, –79, –80, –81, –82, –85, –88, –89, –90, –91, –92, –96, –97, –98, –100, –102, –103, and –104) was higher than the cultivated parent, which could be due to the probable introgression of genes and alleles conferring heat tolerance from the wild *P. violaceum* accession, IP 21544. In Pop 4, though nine ILs, PBPMPOP 4–157, –160, –168, –170, –174, –175, –176, –178, and –195, had ≥70% overall mean seed set across three locations, none of these IL exceeded the heat tolerance level of the cultivated parent. Hence, there is no evidence of transgressive segregation for heat tolerance in this population. The results indicate the probable flowering-stage heat tolerance in wild *Pennisetum* accession, IP 21544. The results also indicated that selecting donor parents based on their availability or adaptation to any specific environment, such as harsh environments of western Africa in this study, cannot be the sole criterion to select donor for prebreeding work. This emphasizes the importance of precise phenotyping of crop wild relatives in order to identify the potential donor for further use in prebreeding programs. Overall, the results show that the 18 ILs from Pop 3 had higher seed set than the recurrent cultivated parent ICMB 94555. As these ILs are derived from wild *P. violaceum* accession, it can be inferred that these ILs may carry novel heat-tolerant genes and alleles, which may be different from the genes and alleles present in the cultivated gene pool. However, detailed study is needed to investigate the level and genetics of heat tolerance in wild *Pennisetum* species, and the expression of this trait with the genes and alleles introgressed from *P. violaceum* in different cultivated backgrounds.

Because of unexpected rains after flowering both at Hisar and Bawal, it was not be possible to identify terminal drought-tolerant ILs from this study. However, a large genetic variation was observed for important agronomic traits, such as days to 50% flowering, plant height, panicle harvest index, and

dry fodder yield, indicating the presence of enormous variability among ILs for these traits. The presence of enormous variability coupled with high heritability for important traits, such as panicle harvest index, days to 50% flowering, plant height, and grain yield, suggests that selection for these traits will be effective and useful. High heritability for these traits has been reported in previous studies in pearl millet (Bind et al., 2015; Priyanka, Shanthi, Reddy, & Ravindra Reddy, 2019: Kumawat, Sharma, & Sharma, 2019; Pujar, Govindaraj, Gangaprasad, Kanatti, & Shivade, 2020; Singh, Yadav, Yadav, Vart, & Yadav, 2014), which supports the results of this study. The genetic variation found in these ILs is expected to be novel with broad genetic bases as it is derived from wild species. Specifically, the significantly higher dry fodder yield of 19 ILs in Pop 3 and 16 ILs in Pop 4 compared with the respective cultivated parent could be of particular interest to the breeders and can serve as novel source of variability for improving dry fodder yield of cultivated pearl millet. The wild species are usually hardy and robust with respect to vegetative growth, and hence, most of these traits might have contributed to the increased dry fodder yield among ILs.

Isolates Pg 45, Pg 53, Pg 56, Pg 118, and Pg 119, representing five pathotypes of *M. grisea* identified based on the pathogenic variability study of 25 isolates, were reported for use in greenhouse screening of pearl millet lines for blast resistance (Sharma et al., 2013). The donor parents IP 21544 and IP 21720 used in the present study were among the 305 *P. violaceum* accessions screened against these five isolates for the identification of blast resistance sources. IP 21544 was resistant to four isolates, whereas IP 21720 was resistant to all the five isolates based on the mean blast score thus selected for the introgression of blast resistance from wild to cultivated pearl millet. However, more virulent pathotypes have now been identified based on virulence diversity study of *M. grisea* isolates, and pathotype isolates Pg 138, Pg 186, Pg 204, and Pg 232 are presently being used at ICRISAT, Patancheru, India, for the greenhouse screening of pearl millet lines. Therefore, ILs derived from the four populations were screened against these new pathotype isolates along with Pg 45 (local isolate) for blast resistance.

Within accession variability for the trait of interest is expected in the germplasm accessions and the ILs, and the same was observed in most of the ILs screened against different isolates for blast resistance. Therefore, efforts were made to stabilize blast resistance in the ILs by selecting the resistant plants in S_0 screening cycle and rescreening the seedlings raised from selfed seed in the next S_1 and S_2 screening cycles. There was an increase in the recovery of resistant plants in the ILs from different populations with the selection of resistant plants and rescreening the selfed seed of resistant plants in the next generation. Overall, the percentage resistant plants observed in all the four populations against all the five blast isolates were 37% in S_0, which increased to 64% in S_1, and the recovery of resistant plants within ILs was further increased to 78% in S_2 generation. However, a slight decrease in percentage resistant plants in some of the selections from some of the ILs was also observed in S_2 vs. S_1. This indicated that these selections were still segregating for blast resistance and need to be stabilized by further selection, selfing, and rescreening of next generation against test isolates of *M. grisea*. A reasonable number of lines showing 100% resistant plants in both S_1 and S_2, maximum being from Pop 2, were observed. Some lines with stable resistance in all the three generations, S_0, S_1, and S_2, with no segregation for blast reaction, were also observed (Supplemental Table S3). Multiple pathotype resistance was also observed in some ILs as the single-plant selections from the same ILs exhibited resistance to two to three pathotype isolates. Maximum ILs exhibiting multiple-pathotype resistance were derived from Pop 2 and 4. Such lines with stable multiple-pathotype resistance hold promise in the transfer of blast resistance from the wild relative to cultivated pearl millet.

A minimum number of resistant plants in the ILs were observed against Pg 138, which is expected, as Pg 138 belongs to the most virulent pathogenic group or pathotype identified so far in the pearl millet infecting populations of *M. grisea*. One selection from Pop 2, PBPMPOP 2-46-1-9 recorded 100% resistant plants in S_2 generation against Pg 138. Similarly, selections from another five ILs from Pop 2 recorded >50% resistant plants in S_2 generation. Efforts are being made to stabilize this resistance by further selection and screening the next one to two generations. All the parents, both recipients and donor, used to develop four populations screened in this study were susceptible to Pg 138. The resistant plants observed against Pg 138 in the ILs indicate the recombination of residual variability for resistance in the parents. Earlier, inbred progenies with high levels of downy mildew resistance have been reported to be developed from a highly downy mildew susceptible line 7042 by pedigree selection for five generations. Singh, Williams, and Reddy (1988) also suggested that hidden residual variability for resistance can be used to improve susceptible hybrids (though commercially promising), which would otherwise be discarded because of their susceptibility. Parents of Pop 1 and Pop 2 (both recipient and donor) were either resistant or moderately resistant (based on mean blast score of seedlings) to pathotype isolates except Pg 138. Hence, the resistance observed in the ILs could be the result of recombination of resistance alleles from both the parents, whereas, recipient parents ICMB 94555 and ICMB 97111 of Pop 3 and Pop 4 were susceptible to all the five isolates. Therefore, resistance in the ILs of these populations must have been introgressed from the wild donor parents. As segregation for blast reaction was observed in some parents to specific pathotype isolates, it would be prudent to first stabilize the parents for blast reaction (resistant or susceptible)

for further prebreeding efforts to introgress blast resistance against evolving virulence of *M. grisea* from wild relatives to cultivated pearl millet.

Overall, a few ILs, such as PBPMPOP 3–80, having improved seed set under heat stress, high dry fodder yield, and resistance to Pg 204 isolate of blast; PBPMPOP 3–98 with improved seed set under heat stress, high dry fodder yield, and resistance to *M. grisea* isolate Pg 45; PBPMPOP 3–78 with improved seed set under heat stress and resistance to isolate Pg 204; PBPMPOP 4–153 having resistance to isolates Pg 45, Pg 186, and Pg 204; and PBPMPOP 4–122, –118, –192, –155, and –120 et al., having resistance to at least two pathotype isolates were identified as promising sources for multiple traits. These newly identified ILs are available in low-yielding genetic backgrounds and having less-desirable plant types at present because of involvement of wild germplasm (*P. violaceum*) in their parentage. Breeders can use these potential new sources of germplasm to improve flowering-period heat tolerance, enhance forage potential, and blast resistance in pearl millet breeding programs in future.

5 | SUMMARY AND CONCLUSION

Heat stress (≥40 ˚C) during flowering time in summer crop and terminal drought stress in the rainy season adversely affects grain yield. In addition, blast disease has emerged as a major biotic stress adversely affecting pearl millet production. Two blast-resistant accessions of wild relative of pearl millet, *P. violaceum*, were used to introgress blast resistance in the cultivated background through advanced backcross method. These accessions originated from the regions experiencing more drought and heat stress than the regions where cultivated pearl millet is grown and, hence, were expected to contribute heat and drought stress tolerance. The evaluation of introgressions lines derived from these two wild accessions for heat stress led to the identification of 18 ILs from Pop 3 with improved seed set compared with the cultivated parent though the heat tolerance in these 18 ILs was not better than the heat-tolerant checks. Further, because of unexpected rains during the grain-filling stage across two locations, Hisar and Bawal, it was difficult to identify drought-tolerant ILs. In this study, 19 ILs from Pop 3 and 16 ILs from Pop 4 showed significantly higher dry fodder yield than the cultivated parent under the rainfed conditions. Besides this, a few ILs were stabilized for pathotype-specific blast resistance. Some ILs were found promising for multiple traits such as improved seed set under heat stress, high dry fodder yield, and resistance to multiple pathotypes of blast. Though, these identified ILs are currently in low-yielding backgrounds because of involvement of wild germplasm, these ILs can be readily used in pearl millet breeding programs to develop parental lines with desired plant types to diversify the genetic base of cultivated pearl millet for heat, dry fodder yield, and blast resistance. The results also indicated the importance of precise phenotyping of wild species to identify the potential donor with high frequency of useful genes and alleles for the target trait prior to involving them in prebreeding work.

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CONFLICT OF INTEREST

Authors have no conflict of interest.

AUTHOR CONTRIBUTIONS

SS, RS, SKG planned the study; SS developed prebreeding populations; RS screened the populations for blast resistance; SKG coordinated the field evaluation for heat and drought across locations; DY and YY evaluated prebreeding populations for drought tolerance at Hisar and Bawal, respectively; RSM, IS, BV evaluated populations for heat tolerance at SK Nagar; YV, VSD evaluated populations for heat stress at Agra; AKJ evaluated population for heat stress at Tharad; SKG and AR analyzed data from drought screening trials; SKG, SS and MP analyzed heat screening data; SS, MP and RS prepared the initial draft; all authors provided their inputs and approved the manuscript.

REFERENCES

Adhikari, U., Nejadhashemi, A. P., & Woznicki, S. A. (2015). Climate change and eastern Africa: A review of impact on major crops. *Food and Energy Security*, *4*, 110–132. https://doi.org/10.1002/fes3.61

Basetti, P., & Westgate, M. E. (1993). Water deficit affects receptivity of maize silks. *Crop Science*, *33*, 279–282. https://doi.org/10.2135/cropsci1993.0011183X003300020013x.

Bind, H., Bharti, B., Kumar, S., Pandey, M., Kumar, D., & Vishwakarma, D. N. (2015). Studies on genetic variability, for fodder yield and its contributing characters in bajra [*Pennisetum glaucum* (L.) r. Br.]. *Agricultural Science Digest*, *35*, 78–80. https://doi.org/10.5958/0976-0547.2015.00017.8

Directorate of Millet Development. (2020). Crop profile. Retrieved from http://www.aicpmip.res.in/aboutus.html

Dujardin, M., & Hanna, W. W. (1989). Developing apomictic pearl millet-characterization of a BC3 plant. *Journal of Genetics and Plant Breeding*, *43*, 145–151.

Gupta, S. K., Rai, K. N., Singh, P., Ameta, V. L., Gupta, S. K., Jayalekha, A. K., … Verma, Y. S. (2015). Seed set variability under high temperatures during flowering period in pearl millet (*Pennisetum glaucum* L. (R.) Br.). *Field Crops Research*, *171*, 41–53. https://doi.org/10.1016/j.fcr.2014.11.005

Hanna, W. W., Wells, H. D., & Burton, G. W. (1985). Dominant gene for rust resistance in pearl millet. *Journal of Heredity*, *76*, 134. https://doi.org/10.1093/oxfordjournals.jhered.a110039

IPCC. (2014). Climate change 2014: Synthesis report. In Pachauri R.K., & Meyer L.A., (Eds.), *Contribution of Working Groups I, II And III to the Fifth Assessment Report Of the Intergovernmental Panel on Climate Change*. Geneva, Switzerland: IPCC.

Jauhar, P. P., & Hanna, W. W. (1998). Cytogenetics and genetics of pearl millet. *Advances in Agronomy*, *64*, 1–26.

Kannan, B., Valencia, E., & Altpeter, F. (2013). Interspecific hybridization between elephant grass and pearl millet and selection of hybrids with high-biomass production and enhanced biosafety. Presented at the *Soil Science Society of America 2013 Annual Meeting: Water, Food, Energy & Innovation for a Sustainable World*, Tampa, FL, 3–6 Nov. 2013. Madison,WI: ASA, CSSA, SSSA.

Kaushal, P., Khare, A., Zadoo, S. N., Roy, A. K., Malaviya, D. R., Agrawal, A., … Choubey, R. N. (2008). Sequential reduction of *Pennisetum squamulatum* genome complement in *P. glaucum* ($2n = 28$) × *P. squamulatum* ($2n = 56$) hybrids and their progenies revealed its octoploid status. *Cytologia*, *73*, 151–158. https://doi.org/10.1508/cytologia.73.151

Krishnamurthy, L., Zaman-Allah, M., Purushothaman, R., Irshad, M., & Vadez, V. (2011). Plant biomass productivity under abiotic stresses in SAT agriculture. In M. D. Matovicb (Ed.), *Biomass—Detection, production and usage* (pp. 247–264). London: IntechOpen Ltd. https://doi.org/10.5772/17279

Kumawat, K. R., Sharma, N. K., & Sharma, N. (2019). Genetic variability and character association analysis in pearl millet single cross hybrids under dry conditions of Rajasthan. *Electronic Journal of Plant Breeding*, *10*, 1067–1070.

Mahalakshmi, V., Bidinger, F. R., & Raju, D. S. (1987). Effect of timing of water-deficit on pearl millet (*Pennisetum americanum*). *Field Crops Research*, *15*, 327–339. https://doi.org/10.1016/0378-4290(87)90020-7

Obok, E. E., Aken'Ova, M. E., & Iwo, G. A. (2012). Forage potentials of interspecific hybrids between elephant grass selections and cultivated pearl millet genotypes of Nigerian origin. *Journal of Plant Breeding and Crop Science*, *4*, 136–143.

Otegui, M. E., & Melón, S. (1997). Kernel set and flower synchrony within the ear of maize: I. Sowing date effects. *Crop Science*, *37*, 441–447. https://doi.org/10.2135/cropsci1997.0011183X003700020023x

Payne, R. W., Murray, D. A., Harding, S. A., Baird, D. B., & Soutar, D. M. (2009). *GenStat for Windows (10th Edition) Introduction*. Hemel Hempstead: VSN International.

Prasad, P. V. V., Bheemanahalli, R., & Jagadish, S. V. K. (2017). Field crops and the fear of heat stress- Opportunities, challenges and future directions. *Field Crop Research*, *200*, 114–121. https://doi.org/10.1016/j.fcr.2016.09.024

Priyanka, V., Shanthi, P., Reddy, D. M., & Ravindra Reddy, B. (2019). Genetic variability studies on yield, physiological and nutritional traits in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *International Journal of Current Microbiology and Applied Sciences*, *8*, 501–508. https://doi.org/10.20546/ijcmas.2019.807.061

Pujar, M., Govindaraj, M., Gangaprasad, S., Kanatti, A., & Shivade, H. (2020). Genetic variation and diversity for grain iron, zinc, protein and agronomic traits in advanced breeding lines of pearl millet [*Pennisetum glaucum* (L.) R. Br.] for biofortification breeding. *Genetic Resources and Crop Evolution*, *67*, 2009–2022. https://doi.org/10.1007/s10722-020-00956-x

Rai, K. N., Hash, C. T., Singh, A. K., & Velu, G. (2008). Adaptation and quality traits of a germplasm-derived commercial seed parent of pearl millet. *Plant Genetic Resources Newsletter*, *154*, 20–24.

SAS Institute Inc. (2018). *SAS/STAT 9.2 user's guide*., Cary, NC: SAS Institute Inc.

Sharma, R., Sharma, S., & Gate, V. L. (2020). Tapping *Pennisetum violaceum*, a wild relative of pearl millet (*Pennisetum glaucum*), for resistance to blast (caused by *Magnaporthe grisea*) and rust (caused by *Puccinia substriata* var. indica). *Plant Disease*, *104*, 1487–1491. https://doi.org/10.1094/PDIS-08-19-1602-RE

Sharma, R., Upadhyaya, H. D., Manjunatha, S. V., Rai, K. N., Gupta, S. K., & Thakur, R. P. (2013). Pathogenic variation in the pearl millet blast pathogen, *Magnaporthe grisea* and identification of resistance to diverse pathotypes. *Plant Disease*, *97*, 89–195. https://doi.org/10.1094/PDIS-05-12-0481-RE

Singh, S. D., Williams, R. J., & Reddy, P. M. (1988). Isolation of downy mildew resistant lines from a highly susceptible cultivar of pearl millet. *Indian Phytopathology*, *41*, 450–456. Retrieved from http://oar.icrisat.org/id/eprint/14

Singh, S., Yadav, Y. P., Yadav, H. P., Vart, D., & Yadav, N. (2014). Studies on genetic variability and trait association for grain yield and its components in pearl millet [*Pennisetum glaucum* (L.) R. Br.]. *Forage Research*, *40*, 91–94.

Singh, S., Yadav, Y. P., Yadav, H. P., Vart, D., & Yadav, N. (2016). Morphological characterization of pearl millet hybrids [*Pennisetum glaucum* (L.) R. Br.] and their parents. *African Journal of Agricultural Research*, *11*, 371–378.

Yadav, O. P. (2010). Drought response of pearl millet landrace-based populations and their crosses with elite composites. *Field Crops Research*, *118*, 51–56. https://doi.org/10.1016/j.fcr.2010.04.005

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

SUPPORTING INFORMATION

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