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# Screening of Pearl Millet [*Pennisetum glaucum* (L.) R. Br.] Germplam Lines for Drought Tolerance Based on Morpho-physiological Traits and SSR Markers

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***Authors' contributions***

*This work was carried out in collaboration among all authors. Author MLC performed the experiment. Authors MKT and ST designed the experiment and performed the statistical analysis. Authors RKP and NG helped in experiment during data recording. Author NT wrote the first draft of the manuscript. Authors MKT and PP edited the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

**Aim:** The present study was undertaken to analyze genetic diversity among pearl millet genotypes based on drought linked morpho-physiological and microsatellite markers.
**Study Design:** In the present investigation, 96 pearl millet germplasm lines were screened against drought using different morphological and physiological traits along with SSR markers.
**Place and Duration of the Study:** The present study was conducted at College of Agriculture, Gwalior, Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, M.P., India during July 2019 to December, 2020.
**Methodology:** The study was conducted to record different morphological and physiological traits related to drought tolerance and susceptibility. Thirty five microsatellite markers were also used in laboratory to analyze the variability among pearl millet genotypes under study.

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**Results:** Pearl millet genotypes were grouped according to their morpho-physiological characteristics. Among 35 SSR markers, twenty-two were successfully amplified across all germplasm lines and seven SSR markers were found to be polymorphic and fifteen markers were monomorphic. All seven polymorphic SSR markers were used consequently for amplification of all the 96 germplasm lines. The range of PIC value was 0.0939 to 0.2980 with the average of 0.2274. The highest PIC value was recorded for the markers Xibmsp26 and Xibmsp29 (0.2980), followed by Xibmsp03 (0.2392), Xibmsp29 (0.2392), Xibmsp06 (0.2289) and Xibmsp07 (0.1948) while the lowest for the marker Xibmsp01 (0.0939). The range of major allele frequency value was 0.7604 to 0.9479 with the average of 0.8363. The range of genetic diversity value was 0.0987 to 0.3644 with the average of 0.2665.

**Conclusions:** According to the morpho-physiological data a total of 22 pearl millet genotypes were found to be grouped distantly from rest of the genotypes. These genotypes had shown their drought tolerance bahaviour however, rests of the genotypes were found to be susceptible against drought.

*Keywords: Pearl millet; drought tolerance; genetic diversity; polymorphism; molecular markers.*

## 1. INTRODUCTION

Pearl millet is a $C_4$, annual and diploid species. It belongs to family poaceae. The present legitimately believed name of pearl millet is *Pennisetum glaucum* (L.) R. Br. [1]. It is supposed to have originated from West Africa [2,3] from where it spread into India and other countries. It is cultivated in the arid tropical region and semi-arid areas of Asia and Africa. It is a primary food for most of the countries in these two regions. It is mainly used in poorest countries and by the poorest peoples. So, also known as the "Poor man's cereal crop" [4]. Nutritionally, it is a good resource of energy and high levels of minerals (such as iron, zinc, calcium, magnesium, phosphorous), vitamins, lipids, crude fibres and high-quality protein 9-13% [5].

Pearl millet is one of the most imperative cultivated cereals in the world, ranking six$^{th}$ after rice, wheat, maize, barley and sorghum in terms of area. It is grown on about 30 mha in more than 30 countries. The majority of this area is in Asia (>10 mha), Africa (about 18 mha) and Americas (>2 mha) [6]. In India, pearl millet is the fourth most widely cultivated edible crop after rice, wheat and maize. It occupies 7.48 million hectares with an average production of 9.21 million tonnes and the productivity of 1231 kgha$^{-1}$ during 2017-18 [7].

Drought stress is the most important environmental constraint limiting factor for crop production worldwide [8]. It limits the agricultural production by preventing the crop plants from expressing their full genetic potential [9]. Terminal drought is shown to contribute to the foremost severe yield losses since it affects spikelet establishment and reduces consequently its fertility [10]. Pearl millet is usually fit for cultivation in arid and semi-arid regions of the country [11]. Due to greater influence of environmental conditions on morphological parameters it is essential to embrace molecular markers in the screening of germplasm lines. Molecular markers offer great scope for improving the efficiency of conventional plant breeding. In the case of drought tolerance, availability of markers tightly linked to tolerant genes will help in identifying plants carrying these genes. Microsatellite markers are adjudged as the most effective and reliable DNA markers for such studies due to their abundance in the genome, multi allelism, genome specificity and even distribution, multi-allelism easy detection, high-throughput, highly reproducible and co-dominantly inherited behaviour [12,13,14,15, 16,17,18,19,20,21,22]. Therefore, the use of SSR markers is a precious approach for the diversity analysis among pearl millet genotypes. Understanding the genetic diversity among genotypes based on phenotypic as well as molecular data may be helpful in identification of contrasting parental materials to enhance heterozygosity [23].

It is envisaged that the selection of diverse parents based on drought tolerance would facilitate the development of transgressive segregates including heterotic groups for hybrid crop breeding in the population. However, genetic diversity analysis solely based on phenotypic traits may not be a steadfast gauge of genetic differences as they are influenced by environmental factors. No systematic works on screening of the pearl millet germplasm lines

selected for the present study based on drought tolerance morpgo-physiological traits and gene – linked SSR markers have been reported. Thus in present study an attempt has been made to estimate the extent of genetic diversity present among different pearl millet germplasm lines for drought tolerance by applying morpho-physiological traits and SSR markers.

## 2. MATERIALS AND METHODS

### 2.1 Morpho-physiological Screening

The present study was consisted of 96 genotypes (Table 1) of (*P. glaucum* (L.) R. Br.) with different reactions to drought *viz*: susceptible, and tolerant. The seeds were obtained from College of Agriculture, Gwalior, RVSKVV, Gwalior, India. The experimental material was monitored in randomized block design (RBD) with two replications. The seeds were sown by hand dibbling. Rainfall supplemented mostly the irrigation requirements. However, extra irrigation was given when required. Fortunately, no rainfall was recorded during period between 50-70 days after sowing which was proved supportive for recording drought parameters. The sampling was done at 60 days after sowing (DAS) till maturity. Five plants were randomly selected from each treatment per replication for recording morpho-physiological data. For drought treatment, the 50-day-old plants were non-irrigated for 10 days and data were recorded for various morpho-physiological parameters *viz.,* plant height, root length, shoot length, root-shoot ratio, spike length, spike girth, numbers of tillers per plant, days of 50% heading initiation, 50% flowering, canopy temperature, fresh weight, dry weight, turgid weight, relative water content, saturation water deficit, days to physiological maturity, leaf area, seed density of spike, yield, biological yield and harvest index after 60 days of sowing to efficiently screen drought tolerant and susceptible genotypes.

### 2.2 Molecular Screening

High quality genomic DNA was isolated from young and fresh leaves from 8-10 days old plantlets, by employing Cetyl trimethyl ammonium bromide (CTAB) method with required modifications as suggested by Tiwari et al. [24]. Extracted DNA was analyzed qualitatively and quantitatively using Nanodrop spectrophotometer and was amplified using SSR specific primers in a Thermal Cycler. The optimized PCR reaction mixture contained 1.0 U/µl *Taq* DNA polymerase, 0.25 mM dNTPs and 1.5 1.7 mM $MgCl_2$. Appropriate annealing temperature was kept 57°C. The Agarose gel electrophoresis (1.5 %) was used to separate amplified product of SSR primer.

In the present study, total of 35 SSR markers (Table 2) for drought were used for genetic diversity analysis (Table 2). The sequences of pearl millet specific SSR markers were obtained from primer reported by Sehgal et al. [25]. The SSR markers were scored based on the size of fragments amplified across all pearl millet genotypes. The major allelic frequency, polymorphism information content and genetic distance-based clustering was performed with Unweighted Pair Group Method for Arithmetic Average (UPGMA) tree using power Marker v3.25 software.

## 3. RESULTS

### 3.1 Morpho-physiological Variations among Pearl Millet Genotypes

Analysis of variance was found significant for most of the traits that suggested existence of substantial sum of variability in studied materials for further improvement (Table 1).

#### 3.1.1 Plant height (cm)

Plant heights varied between 188.05 cm and 295.55 cm among 96 different pearl millet genotypes with an average of 237.30 cm. The maximum plant height was exhibited by genotype IP199 (295.55 cm), followed by IP126 (283.40) and IP137 (282.65).

#### 3.1.2 Root length (cm)

Significant genotypic differences were observed for root length between 15.10 cm to 24.50 cm with mean value of 17.81 cm. Maximum root length value was exhibited by genotype IP177 (24.50 cm) followed by IP 169 (23.00 cm) and IP 190 (22.80). While the lowest count was 15.10 cm evidenced with genotype IP139.

#### 3.1.3 Shoot length (cm)

Shoot length differed between 162.15 cm to 273.50 cm, maximum with germplasm IP199 (273.50 cm) intimately tracked by two genotypes *namely*: IP137 (256.90 cm) and IP126 (248.80 cm). While, the lowest value exhibited by the genotype IP236 (162.15 cm).

**Table 1. Mean performance, range and coefficient of variation of different morpho-physiological traits of pearl millet germplasm lines**

| Sr. no. | Genotype | DAS_50 | DAF_50 | CT $^0$C | LA cm | PH cm | SL cm | SG cm | SHL cm | RL cm | RSR | SDS /cm2 | NT | FW gm | DW gm | TW gm | RWC % | SWD | DPM | DM | TSW gm | YLD Qha$^{-1}$ | BYD Qha$^{-1}$ | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | IP 132 | 44.00 | 48.10 | 35.00 | 153.73 | 229.05 | 23.45 | 3.20 | 205.60 | 20.95 | 0.10 | 18.75 | 1.40 | 193.45 | 144.25 | 288.60 | 33.96 | 66.04 | 83.50 | 96.20 | 11.35 | 33.63 | 208.70 | 16.11 |
| 2 | IP 118 | 45.20 | 51.00 | 33.25 | 216.78 | 213.65 | 26.25 | 2.65 | 187.40 | 19.60 | 0.10 | 21.95 | 1.50 | 169.30 | 114.90 | 225.25 | 49.31 | 50.69 | 81.50 | 95.40 | 9.40 | 27.85 | 170.22 | 16.41 |
| 3 | IP 152 | 44.40 | 50.90 | 33.75 | 197.51 | 218.05 | 24.85 | 3.35 | 193.20 | 20.90 | 0.11 | 22.60 | 1.70 | 166.85 | 129.15 | 222.70 | 40.28 | 59.72 | 82.50 | 95.55 | 12.50 | 37.04 | 191.33 | 19.41 |
| 4 | IP 175 | 43.00 | 49.10 | 32.80 | 306.77 | 244.20 | 26.10 | 2.75 | 218.10 | 16.50 | 0.08 | 17.10 | 1.90 | 195.50 | 111.05 | 231.95 | 70.29 | 29.71 | 83.80 | 94.20 | 10.89 | 32.25 | 164.52 | 19.69 |
| 5 | IP 133 | 41.90 | 47.30 | 35.55 | 235.82 | 253.75 | 24.40 | 3.05 | 229.35 | 17.50 | 0.08 | 20.30 | 1.30 | 262.50 | 183.55 | 318.35 | 58.59 | 41.41 | 82.10 | 95.60 | 10.63 | 31.48 | 271.93 | 11.59 |
| 6 | IP 173 | 44.20 | 49.00 | 34.30 | 293.04 | 277.10 | 32.10 | 3.45 | 245.00 | 16.50 | 0.07 | 17.90 | 1.50 | 290.60 | 218.55 | 372.05 | 47.26 | 52.74 | 84.40 | 93.70 | 10.68 | 31.63 | 229.28 | 13.80 |
| 7 | IP 199 | 44.20 | 52.00 | 34.40 | 185.34 | 295.55 | 22.05 | 2.65 | 273.50 | 17.55 | 0.06 | 23.40 | 1.50 | 139.45 | 96.60 | 183.30 | 49.10 | 50.90 | 82.30 | 94.30 | 11.21 | 33.21 | 143.11 | 23.21 |
| 8 | IP 127 | 41.70 | 48.00 | 32.35 | 259.39 | 233.60 | 25.90 | 3.10 | 207.70 | 18.75 | 0.09 | 28.40 | 1.30 | 292.50 | 201.25 | 322.00 | 75.58 | 24.42 | 81.30 | 95.80 | 9.03 | 26.74 | 218.15 | 12.28 |
| 9 | IP 198 | 41.30 | 47.00 | 34.30 | 234.80 | 228.80 | 21.00 | 3.70 | 207.80 | 20.80 | 0.10 | 19.55 | 1.30 | 369.50 | 209.65 | 409.80 | 79.86 | 20.14 | 83.00 | 95.30 | 12.20 | 36.15 | 310.59 | 11.66 |
| 10 | IP 177 | 42.70 | 49.30 | 34.55 | 240.10 | 230.10 | 21.35 | 2.45 | 208.75 | 24.50 | 0.12 | 29.35 | 1.50 | 240.60 | 134.40 | 288.70 | 68.76 | 31.24 | 84.70 | 95.20 | 10.65 | 31.56 | 199.11 | 15.87 |
| 11 | IP 182 | 42.10 | 50.20 | 34.40 | 416.38 | 276.35 | 33.40 | 2.85 | 242.95 | 16.35 | 0.07 | 27.10 | 1.40 | 247.50 | 172.60 | 313.00 | 53.22 | 46.78 | 82.80 | 96.00 | 11.83 | 35.04 | 255.70 | 13.72 |
| 12 | IP 147 | 41.90 | 48.40 | 34.55 | 271.32 | 260.25 | 31.15 | 2.65 | 229.10 | 16.05 | 0.07 | 27.75 | 1.30 | 188.05 | 140.75 | 274.40 | 35.68 | 64.32 | 82.60 | 93.20 | 10.83 | 32.07 | 208.52 | 15.39 |
| 13 | IP 107 | 41.20 | 47.00 | 33.30 | 284.86 | 238.15 | 25.75 | 3.20 | 212.40 | 18.73 | 0.09 | 26.00 | 1.80 | 251.90 | 183.65 | 303.25 | 57.03 | 42.97 | 81.50 | 93.80 | 11.10 | 32.89 | 272.07 | 12.09 |
| 14 | IP 140 | 42.10 | 50.80 | 32.70 | 241.75 | 217.05 | 20.25 | 2.60 | 196.80 | 22.14 | 0.11 | 23.05 | 1.90 | 218.55 | 96.20 | 259.65 | 75.45 | 24.55 | 82.60 | 97.20 | 11.00 | 32.59 | 119.93 | 27.26 |
| 15 | IP 164 | 42.80 | 51.00 | 33.50 | 230.45 | 206.10 | 18.90 | 2.50 | 187.20 | 21.50 | 0.11 | 29.70 | 1.70 | 307.95 | 141.25 | 365.05 | 75.16 | 24.84 | 84.00 | 94.70 | 11.38 | 33.70 | 209.26 | 16.13 |
| 16 | IP 142 | 43.20 | 50.00 | 34.65 | 236.33 | 243.65 | 24.45 | 3.10 | 219.20 | 16.35 | 0.07 | 27.95 | 1.70 | 233.25 | 127.10 | 305.25 | 59.59 | 40.41 | 82.50 | 95.30 | 12.13 | 35.93 | 188.30 | 19.12 |
| 17 | IP 180 | 42.00 | 50.10 | 34.30 | 248.37 | 263.85 | 24.20 | 3.20 | 239.65 | 15.55 | 0.06 | 29.80 | 1.60 | 278.35 | 224.85 | 364.60 | 38.94 | 61.06 | 82.40 | 93.80 | 10.35 | 30.67 | 233.11 | 13.17 |
| 18 | IP 188 | 45.00 | 53.10 | 34.30 | 306.53 | 233.95 | 20.40 | 2.35 | 213.55 | 17.10 | 0.08 | 27.25 | 1.30 | 310.10 | 193.85 | 331.55 | 86.47 | 13.53 | 83.40 | 95.20 | 11.23 | 33.26 | 287.18 | 11.59 |
| 19 | IP 181 | 41.70 | 51.00 | 34.70 | 254.15 | 256.50 | 30.40 | 2.50 | 226.10 | 19.50 | 0.09 | 29.25 | 1.50 | 280.65 | 190.05 | 311.05 | 75.39 | 24.61 | 83.30 | 96.30 | 11.53 | 34.15 | 281.56 | 12.13 |
| 20 | IP 129 | 43.90 | 50.80 | 33.40 | 382.95 | 234.65 | 17.95 | 3.10 | 216.70 | 16.45 | 0.08 | 26.10 | 1.80 | 210.60 | 139.00 | 284.65 | 49.26 | 50.74 | 83.20 | 95.30 | 9.93 | 29.41 | 205.93 | 14.28 |
| 21 | IP 119 | 41.90 | 49.80 | 33.70 | 142.07 | 250.10 | 30.40 | 2.45 | 219.70 | 17.35 | 0.08 | 19.10 | 1.60 | 210.95 | 131.90 | 408.75 | 28.80 | 71.20 | 80.90 | 93.20 | 11.66 | 34.53 | 195.41 | 17.70 |
| 22 | IP 150 | 41.90 | 48.20 | 35.90 | 255.55 | 280.25 | 27.60 | 2.85 | 252.65 | 17.80 | 0.07 | 23.05 | 1.40 | 288.10 | 209.35 | 369.10 | 49.45 | 50.55 | 83.30 | 94.90 | 10.10 | 29.93 | 222.65 | 13.47 |
| 23 | IP 120 | 42.40 | 51.10 | 35.15 | 245.81 | 268.20 | 24.90 | 3.05 | 243.30 | 16.80 | 0.07 | 30.05 | 1.70 | 250.65 | 159.05 | 293.35 | 68.88 | 31.12 | 83.60 | 95.60 | 11.72 | 34.73 | 250.44 | 14.02 |
| 24 | IP 111 | 42.10 | 49.00 | 35.10 | 205.99 | 244.55 | 16.80 | 2.60 | 227.75 | 15.76 | 0.07 | 27.75 | 1.60 | 189.65 | 98.70 | 228.15 | 70.91 | 29.09 | 82.50 | 95.70 | 11.40 | 33.78 | 126.22 | 26.76 |
| 25 | IP 160 | 43.30 | 52.00 | 36.45 | 237.85 | 232.55 | 20.20 | 2.45 | 212.35 | 18.85 | 0.09 | 22.05 | 1.90 | 180.85 | 113.65 | 214.50 | 68.07 | 31.93 | 82.50 | 94.70 | 9.15 | 27.11 | 168.37 | 16.16 |
| 26 | IP 136 | 44.90 | 51.20 | 35.35 | 202.22 | 233.60 | 25.95 | 2.50 | 207.65 | 16.50 | 0.08 | 26.75 | 1.50 | 190.30 | 125.75 | 229.00 | 61.69 | 38.31 | 80.80 | 95.00 | 11.46 | 33.94 | 186.30 | 18.27 |
| 27 | IP 171 | 42.85 | 52.90 | 34.95 | 278.73 | 199.55 | 24.75 | 2.70 | 174.80 | 17.95 | 0.10 | 21.30 | 1.70 | 176.55 | 119.40 | 228.85 | 52.48 | 47.52 | 82.00 | 93.40 | 11.42 | 33.82 | 176.89 | 19.14 |
| 28 | IP 130 | 43.00 | 51.30 | 37.15 | 205.04 | 226.10 | 30.40 | 2.60 | 195.70 | 18.45 | 0.09 | 22.45 | 1.30 | 209.45 | 108.30 | 260.85 | 66.60 | 33.40 | 84.00 | 93.30 | 10.65 | 31.56 | 160.44 | 19.67 |
| 29 | IP 166 | 44.70 | 52.00 | 36.00 | 153.68 | 229.70 | 36.50 | 2.30 | 193.20 | 16.70 | 0.09 | 23.60 | 1.40 | 179.35 | 72.05 | 214.85 | 75.55 | 24.45 | 83.50 | 95.80 | 12.57 | 37.23 | 106.56 | 35.03 |
| 30 | IP 128 | 41.70 | 50.10 | 36.05 | 282.26 | 265.60 | 22.90 | 2.45 | 242.70 | 16.35 | 0.07 | 23.30 | 1.50 | 183.65 | 108.60 | 249.60 | 57.09 | 42.91 | 82.50 | 95.70 | 10.98 | 32.52 | 160.89 | 20.21 |
| 31 | IP 183 | 44.50 | 50.90 | 36.95 | 266.63 | 236.20 | 30.20 | 2.55 | 206.00 | 20.85 | 0.10 | 22.90 | 1.70 | 149.45 | 96.05 | 188.55 | 58.91 | 41.09 | 84.70 | 94.20 | 12.28 | 36.39 | 142.30 | 25.73 |
| 32 | IP 165 | 42.00 | 53.10 | 35.30 | 229.15 | 229.45 | 23.40 | 2.55 | 206.05 | 17.20 | 0.08 | 22.75 | 1.40 | 220.50 | 159.80 | 258.60 | 63.93 | 36.07 | 82.80 | 96.00 | 12.95 | 38.37 | 236.74 | 16.25 |
| 33 | IP 192 | 43.70 | 51.10 | 33.70 | 395.73 | 248.55 | 25.10 | 2.65 | 223.45 | 15.65 | 0.07 | 27.25 | 1.50 | 142.45 | 69.70 | 178.00 | 67.01 | 32.99 | 81.50 | 96.90 | 12.58 | 37.26 | 103.26 | 36.20 |

| Sr. no. | Genotype | DAS_50 | DAF_50 | CT $^0$C | LA cm | PH cm | SL cm | SG cm | SHL cm | RL cm | RSR | SDS /cm2 | NT | FW gm | DW gm | TW gm | RWC % | SWD | DPM | DM | TSW gm | YLD Qha$^{-1}$ | BYD Qha$^{-1}$ | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 34 | IP 122 | 44.20 | 51.10 | 34.55 | 333.11 | 261.55 | 25.60 | 2.70 | 235.95 | 16.55 | 0.07 | 22.45 | 1.80 | 158.30 | 99.30 | 202.90 | 56.95 | 43.05 | 81.50 | 95.70 | 10.75 | 31.85 | 147.11 | 21.65 |
| 35 | IP 143 | 42.10 | 53.10 | 32.95 | 210.47 | 237.05 | 29.65 | 2.35 | 207.40 | 18.85 | 0.09 | 22.80 | 1.80 | 149.60 | 55.10 | 184.90 | 73.32 | 26.68 | 84.50 | 96.30 | 10.52 | 31.17 | 81.04 | 38.87 |
| 36 | IP 167 | 45.00 | 52.00 | 33.10 | 190.12 | 217.50 | 23.70 | 3.30 | 193.80 | 17.45 | 0.09 | 22.15 | 1.40 | 147.40 | 85.35 | 203.45 | 53.77 | 46.23 | 84.40 | 94.40 | 12.42 | 36.79 | 126.44 | 29.15 |
| 37 | IP 172 | 42.20 | 50.00 | 34.50 | 257.47 | 238.75 | 29.00 | 3.05 | 209.75 | 15.85 | 0.08 | 25.30 | 1.50 | 152.80 | 90.55 | 199.60 | 60.26 | 39.74 | 82.40 | 97.00 | 11.70 | 34.67 | 134.15 | 25.85 |
| 38 | IP 106 | 42.70 | 53.10 | 33.00 | 278.32 | 244.35 | 23.80 | 3.25 | 220.55 | 18.60 | 0.08 | 25.30 | 1.50 | 149.30 | 90.65 | 201.90 | 52.89 | 47.11 | 84.10 | 92.30 | 11.49 | 34.03 | 134.30 | 25.40 |
| 39 | IP 137 | 42.30 | 52.00 | 32.15 | 341.59 | 282.65 | 25.75 | 3.10 | 256.90 | 16.80 | 0.07 | 23.30 | 1.50 | 178.60 | 103.10 | 225.30 | 63.89 | 36.11 | 82.80 | 93.20 | 9.55 | 28.30 | 152.74 | 18.52 |
| 40 | IP 116 | 44.30 | 50.30 | 32.65 | 224.40 | 241.45 | 26.20 | 2.60 | 215.25 | 15.25 | 0.07 | 27.00 | 1.50 | 124.75 | 65.15 | 169.90 | 57.78 | 42.22 | 82.20 | 96.10 | 10.93 | 32.37 | 96.52 | 33.56 |
| 41 | IP 194 | 44.40 | 51.00 | 35.85 | 180.33 | 242.30 | 30.40 | 2.50 | 211.90 | 21.90 | 0.10 | 21.05 | 1.60 | 121.75 | 62.25 | 159.85 | 61.18 | 38.82 | 83.40 | 91.80 | 10.78 | 31.93 | 92.22 | 34.73 |
| 42 | IP 195 | 42.90 | 49.10 | 32.35 | 228.30 | 223.55 | 22.80 | 2.70 | 200.75 | 20.55 | 0.10 | 25.95 | 1.50 | 267.40 | 107.40 | 301.30 | 82.45 | 17.55 | 83.50 | 93.70 | 12.43 | 36.81 | 153.93 | 23.93 |
| 43 | IP 126 | 41.50 | 49.50 | 31.90 | 211.80 | 283.40 | 34.60 | 2.55 | 248.80 | 21.80 | 0.09 | 21.05 | 1.80 | 297.45 | 145.85 | 334.60 | 80.45 | 19.55 | 82.70 | 95.80 | 10.90 | 32.30 | 253.11 | 13.15 |
| 44 | IP 155 | 43.00 | 54.10 | 33.10 | 222.62 | 268.60 | 40.90 | 3.25 | 227.70 | 15.20 | 0.07 | 24.80 | 2.00 | 332.65 | 269.25 | 377.30 | 59.19 | 40.81 | 83.00 | 95.60 | 9.85 | 29.19 | 218.89 | 13.36 |
| 45 | IP 149 | 43.00 | 53.10 | 33.15 | 260.87 | 229.05 | 31.70 | 3.60 | 197.35 | 20.15 | 0.10 | 22.45 | 1.70 | 257.05 | 189.95 | 316.05 | 53.25 | 46.75 | 83.40 | 95.50 | 10.60 | 31.41 | 281.41 | 11.16 |
| 46 | IP 185 | 41.70 | 49.80 | 32.15 | 349.12 | 238.45 | 27.00 | 3.25 | 211.45 | 16.80 | 0.08 | 25.85 | 1.50 | 159.50 | 93.85 | 210.15 | 56.43 | 43.57 | 86.00 | 91.70 | 12.33 | 36.52 | 139.04 | 26.28 |
| 47 | IP 161 | 42.90 | 50.80 | 31.95 | 236.28 | 234.10 | 19.90 | 2.60 | 214.20 | 17.55 | 0.08 | 21.50 | 1.60 | 228.55 | 167.60 | 283.20 | 52.74 | 47.26 | 84.50 | 94.80 | 12.00 | 35.56 | 248.30 | 14.33 |
| 48 | IP 168 | 44.10 | 51.00 | 31.95 | 325.45 | 251.60 | 27.80 | 2.35 | 223.80 | 19.45 | 0.09 | 20.05 | 1.70 | 211.50 | 109.25 | 248.05 | 73.85 | 26.15 | 86.00 | 94.50 | 10.29 | 30.47 | 161.85 | 18.91 |
| 49 | IP 190 | 43.00 | 50.20 | 31.95 | 371.93 | 266.40 | 27.45 | 2.85 | 238.95 | 22.80 | 0.10 | 22.10 | 1.70 | 268.85 | 136.20 | 304.10 | 79.02 | 20.98 | 83.70 | 94.60 | 11.63 | 34.44 | 201.78 | 17.23 |
| 50 | IP 156 | 43.30 | 53.10 | 32.05 | 266.25 | 222.50 | 21.05 | 2.45 | 201.45 | 16.90 | 0.08 | 19.00 | 1.60 | 173.80 | 95.85 | 207.15 | 70.20 | 29.80 | 84.40 | 93.20 | 13.28 | 39.33 | 142.00 | 27.94 |
| 51 | IP 187 | 41.90 | 49.90 | 32.65 | 377.77 | 223.25 | 27.25 | 3.10 | 196.00 | 15.70 | 0.08 | 20.35 | 1.30 | 200.15 | 121.85 | 252.15 | 59.70 | 40.30 | 84.60 | 96.20 | 11.68 | 34.59 | 180.52 | 19.19 |
| 52 | IP 159 | 41.10 | 48.00 | 32.15 | 217.63 | 210.00 | 16.40 | 2.60 | 193.60 | 15.30 | 0.08 | 28.25 | 1.50 | 300.05 | 187.95 | 343.30 | 72.56 | 27.44 | 85.90 | 95.50 | 12.25 | 36.30 | 278.44 | 13.13 |
| 53 | IP 139 | 42.60 | 50.90 | 32.45 | 303.90 | 215.25 | 27.45 | 2.55 | 187.80 | 15.10 | 0.08 | 23.60 | 1.40 | 142.15 | 73.15 | 199.00 | 54.79 | 45.21 | 81.90 | 96.40 | 12.17 | 36.06 | 108.37 | 33.34 |
| 54 | IP 146 | 42.80 | 51.20 | 32.55 | 290.48 | 238.45 | 27.45 | 2.55 | 211.00 | 15.80 | 0.07 | 27.15 | 1.70 | 173.10 | 120.55 | 215.60 | 55.96 | 44.04 | 83.60 | 94.80 | 12.30 | 36.44 | 178.59 | 20.41 |
| 55 | IP 196 | 45.30 | 53.20 | 33.30 | 232.25 | 206.10 | 18.70 | 2.70 | 187.40 | 16.80 | 0.09 | 19.90 | 1.80 | 212.45 | 93.50 | 245.75 | 78.19 | 21.81 | 82.10 | 97.80 | 10.44 | 30.92 | 138.52 | 22.65 |
| 56 | IP 186 | 42.30 | 50.80 | 32.70 | 241.01 | 232.00 | 23.05 | 2.50 | 208.95 | 19.10 | 0.09 | 23.80 | 1.80 | 113.60 | 61.50 | 165.10 | 49.91 | 50.09 | 83.40 | 93.30 | 9.93 | 29.41 | 91.11 | 32.41 |
| 57 | IP 158 | 45.20 | 53.30 | 33.50 | 220.81 | 265.45 | 24.75 | 2.35 | 240.70 | 22.20 | 0.09 | 23.10 | 1.40 | 232.60 | 142.00 | 275.25 | 68.28 | 31.72 | 83.00 | 94.60 | 12.03 | 35.63 | 210.37 | 16.96 |
| 58 | IP 151 | 42.70 | 50.20 | 34.20 | 182.75 | 228.95 | 30.25 | 2.45 | 198.70 | 15.90 | 0.08 | 23.90 | 1.40 | 203.75 | 144.65 | 253.40 | 54.97 | 45.03 | 82.00 | 94.60 | 11.40 | 33.78 | 214.30 | 15.78 |
| 59 | IP 193 | 43.10 | 51.00 | 33.25 | 299.78 | 258.65 | 19.10 | 2.60 | 239.55 | 17.20 | 0.07 | 22.00 | 1.50 | 221.55 | 159.10 | 289.65 | 47.35 | 52.65 | 85.80 | 97.00 | 11.28 | 33.41 | 235.70 | 14.23 |
| 60 | IP 105 | 43.10 | 52.80 | 31.70 | 190.16 | 207.55 | 27.85 | 2.60 | 179.70 | 16.65 | 0.09 | 23.60 | 1.50 | 170.25 | 107.75 | 217.70 | 57.11 | 42.89 | 87.70 | 95.20 | 10.30 | 30.52 | 159.63 | 19.13 |
| 61 | IP 123 | 42.00 | 50.30 | 31.85 | 232.94 | 243.80 | 19.25 | 3.20 | 224.55 | 17.65 | 0.08 | 24.05 | 1.50 | 214.35 | 139.65 | 267.25 | 59.31 | 40.69 | 82.50 | 95.70 | 12.40 | 36.74 | 206.89 | 17.97 |
| 62 | IP 131 | 43.10 | 50.80 | 33.40 | 184.01 | 232.25 | 24.00 | 2.60 | 208.25 | 15.80 | 0.08 | 24.75 | 1.30 | 220.70 | 124.10 | 259.60 | 75.60 | 24.40 | 83.00 | 94.80 | 12.58 | 37.26 | 183.85 | 20.34 |
| 63 | IP 178 | 41.90 | 50.80 | 33.70 | 301.23 | 246.25 | 25.90 | 2.75 | 220.35 | 21.90 | 0.10 | 24.30 | 1.70 | 181.75 | 118.35 | 209.90 | 69.12 | 30.88 | 83.40 | 94.00 | 11.00 | 32.59 | 175.33 | 18.60 |
| 64 | IP 121 | 43.40 | 52.10 | 33.40 | 189.06 | 214.05 | 22.85 | 2.70 | 191.20 | 19.70 | 0.10 | 21.85 | 1.30 | 209.55 | 87.90 | 279.80 | 63.85 | 36.15 | 83.50 | 95.00 | 10.45 | 30.95 | 145.04 | 22.32 |
| 65 | IP 104 | 42.90 | 53.10 | 33.15 | 261.40 | 233.55 | 28.65 | 2.50 | 204.90 | 15.90 | 0.08 | 20.50 | 1.90 | 372.45 | 234.25 | 422.65 | 73.35 | 26.65 | 85.50 | 94.70 | 10.20 | 30.22 | 247.04 | 12.25 |
| 66 | IP 134 | 44.60 | 50.70 | 33.50 | 243.74 | 225.35 | 26.40 | 2.50 | 198.95 | 17.60 | 0.09 | 20.00 | 1.60 | 261.55 | 190.25 | 300.75 | 64.54 | 35.46 | 84.50 | 94.80 | 10.53 | 31.19 | 281.85 | 11.08 |
| 67 | IP 112 | 42.60 | 50.90 | 33.00 | 160.83 | 237.75 | 27.30 | 2.30 | 210.45 | 15.65 | 0.07 | 23.65 | 1.50 | 301.25 | 160.55 | 338.25 | 78.99 | 21.01 | 83.50 | 91.30 | 11.15 | 33.04 | 237.85 | 13.92 |
| 68 | IP 141 | 44.30 | 49.40 | 33.55 | 283.54 | 221.00 | 23.30 | 2.20 | 197.70 | 15.30 | 0.08 | 25.10 | 1.40 | 259.55 | 188.30 | 306.75 | 60.53 | 39.47 | 86.50 | 98.40 | 8.98 | 26.59 | 223.96 | 11.87 |

| Sr. no. | Genotype | DAS_50 | DAF_50 | CT $^0$C | LA cm | PH cm | SL cm | SG cm | SHL cm | RL cm | RSR | SDS /cm2 | NT | FW gm | DW gm | TW gm | RWC % | SWD | DPM | DM | TSW gm | YLD Qha$^{-1}$ | BYD Qha$^{-1}$ | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 69 | IP 145 | 41.90 | 48.90 | 31.65 | 192.92 | 246.65 | 19.05 | 2.35 | 227.60 | 15.15 | 0.07 | 21.00 | 1.50 | 257.10 | 178.05 | 326.85 | 53.76 | 46.24 | 82.00 | 97.10 | 9.65 | 28.59 | 263.78 | 10.84 |
| 70 | IP 144 | 42.10 | 51.10 | 33.60 | 373.71 | 213.60 | 31.75 | 2.65 | 181.85 | 18.05 | 0.10 | 19.80 | 1.50 | 176.10 | 118.75 | 233.50 | 50.07 | 49.93 | 85.40 | 94.40 | 8.75 | 25.93 | 175.93 | 14.73 |
| 71 | IP 138 | 41.60 | 49.80 | 33.55 | 303.53 | 233.25 | 27.35 | 2.60 | 205.90 | 16.10 | 0.08 | 22.75 | 1.40 | 234.65 | 123.20 | 266.00 | 78.11 | 21.89 | 83.40 | 93.80 | 10.59 | 31.38 | 182.52 | 17.33 |
| 72 | IP 179 | 43.40 | 50.40 | 32.80 | 271.07 | 247.25 | 31.40 | 2.70 | 215.85 | 18.15 | 0.08 | 18.95 | 1.50 | 182.90 | 126.80 | 224.45 | 57.71 | 42.29 | 82.10 | 96.70 | 12.03 | 35.63 | 187.85 | 18.97 |
| 73 | IP 153 | 42.20 | 49.70 | 34.05 | 290.74 | 213.55 | 25.75 | 2.55 | 187.80 | 16.50 | 0.09 | 23.80 | 1.70 | 112.35 | 68.15 | 146.15 | 56.57 | 43.43 | 87.40 | 95.80 | 12.18 | 36.07 | 100.96 | 35.78 |
| 74 | IP 101 | 41.90 | 48.80 | 32.45 | 241.13 | 220.55 | 27.10 | 3.05 | 193.45 | 15.70 | 0.08 | 25.65 | 1.30 | 139.70 | 93.95 | 177.70 | 55.36 | 44.64 | 82.90 | 94.20 | 9.88 | 29.26 | 139.19 | 21.02 |
| 75 | IP 135 | 42.60 | 51.50 | 33.45 | 230.61 | 202.25 | 25.40 | 2.55 | 176.85 | 16.25 | 0.09 | 27.90 | 1.70 | 228.75 | 150.90 | 276.00 | 62.24 | 37.76 | 85.90 | 92.90 | 10.45 | 30.96 | 223.56 | 13.88 |
| 76 | IP 162 | 41.40 | 49.80 | 34.05 | 250.38 | 238.85 | 24.60 | 2.55 | 214.25 | 17.50 | 0.08 | 24.60 | 1.50 | 183.50 | 96.05 | 222.00 | 69.48 | 30.52 | 84.50 | 95.20 | 11.15 | 33.04 | 142.30 | 23.36 |
| 77 | IP 115 | 43.90 | 53.00 | 34.65 | 279.53 | 207.30 | 26.95 | 2.40 | 180.35 | 17.05 | 0.09 | 22.75 | 1.40 | 131.25 | 85.65 | 169.20 | 54.30 | 45.70 | 85.50 | 97.50 | 11.58 | 34.30 | 126.89 | 27.08 |
| 78 | IP 170 | 42.50 | 56.10 | 36.10 | 240.57 | 223.80 | 38.70 | 3.30 | 185.10 | 15.25 | 0.08 | 24.85 | 1.60 | 214.75 | 148.60 | 253.80 | 62.85 | 37.15 | 85.40 | 96.60 | 9.40 | 27.85 | 220.15 | 12.66 |
| 79 | IP 109 | 43.00 | 53.10 | 33.50 | 220.15 | 258.35 | 18.55 | 2.45 | 239.80 | 21.90 | 0.09 | 24.55 | 1.20 | 260.85 | 165.75 | 307.35 | 67.16 | 32.84 | 88.40 | 93.80 | 13.23 | 39.19 | 245.56 | 15.98 |
| 80 | IP 154 | 45.50 | 53.90 | 33.00 | 361.75 | 212.25 | 25.75 | 3.10 | 186.50 | 21.25 | 0.11 | 24.95 | 2.00 | 200.65 | 123.10 | 239.95 | 66.36 | 33.64 | 83.40 | 94.10 | 9.98 | 29.56 | 182.37 | 16.21 |
| 81 | IP 174 | 43.30 | 51.00 | 32.15 | 181.59 | 248.55 | 34.80 | 2.35 | 213.75 | 15.25 | 0.07 | 23.10 | 1.60 | 151.95 | 114.05 | 190.95 | 49.78 | 50.22 | 82.00 | 95.50 | 9.95 | 29.48 | 168.96 | 17.48 |
| 82 | IP 108 | 41.40 | 48.20 | 32.45 | 198.51 | 237.05 | 25.05 | 2.65 | 212.00 | 18.20 | 0.09 | 19.85 | 1.70 | 138.70 | 107.60 | 186.25 | 39.54 | 60.46 | 83.50 | 90.90 | 8.93 | 26.44 | 159.41 | 16.59 |
| 83 | IP 189 | 42.70 | 50.70 | 32.40 | 261.49 | 260.60 | 27.35 | 2.45 | 233.25 | 15.95 | 0.07 | 24.25 | 1.50 | 177.40 | 98.25 | 223.15 | 63.58 | 36.42 | 85.10 | 93.90 | 10.35 | 30.67 | 145.56 | 21.12 |
| 84 | IP 110 | 43.00 | 48.10 | 33.75 | 287.12 | 231.40 | 24.25 | 3.05 | 207.15 | 17.00 | 0.08 | 23.50 | 1.50 | 253.45 | 144.00 | 297.30 | 71.42 | 28.58 | 82.50 | 96.60 | 11.65 | 34.52 | 213.33 | 16.20 |
| 85 | IP 117 | 44.00 | 51.00 | 32.90 | 204.54 | 230.25 | 17.10 | 2.20 | 213.15 | 16.30 | 0.08 | 20.65 | 1.60 | 191.35 | 92.50 | 230.50 | 71.61 | 28.39 | 87.40 | 95.70 | 11.77 | 34.86 | 137.04 | 25.45 |
| 86 | IP 169 | 41.40 | 50.00 | 34.15 | 186.08 | 244.55 | 23.05 | 2.55 | 221.50 | 23.00 | 0.10 | 23.65 | 1.50 | 127.65 | 73.05 | 165.20 | 60.59 | 39.41 | 80.80 | 97.00 | 9.30 | 27.56 | 108.22 | 25.49 |
| 87 | IP 114 | 43.80 | 52.00 | 33.05 | 209.13 | 229.95 | 24.25 | 2.40 | 205.70 | 18.20 | 0.09 | 19.75 | 1.90 | 228.50 | 100.55 | 263.65 | 77.93 | 22.07 | 83.50 | 97.30 | 9.98 | 29.56 | 128.96 | 22.97 |
| 88 | IP 163 | 43.80 | 52.80 | 33.35 | 206.97 | 245.75 | 34.55 | 2.35 | 211.20 | 15.70 | 0.07 | 23.25 | 1.50 | 158.10 | 76.75 | 199.05 | 66.49 | 33.51 | 85.40 | 95.50 | 10.23 | 30.30 | 113.70 | 27.08 |
| 89 | IP 274 | 42.70 | 51.20 | 34.50 | 271.18 | 232.75 | 31.35 | 2.80 | 201.40 | 16.60 | 0.08 | 24.20 | 1.60 | 180.50 | 106.20 | 226.65 | 61.95 | 38.05 | 85.60 | 95.80 | 10.49 | 31.08 | 157.33 | 19.77 |
| 90 | IP 283 | 43.00 | 50.90 | 34.40 | 200.01 | 238.55 | 21.20 | 2.55 | 217.35 | 17.00 | 0.08 | 21.05 | 1.40 | 180.45 | 149.40 | 251.85 | 30.59 | 69.41 | 84.40 | 95.00 | 10.60 | 31.41 | 221.33 | 14.19 |
| 91 | IP 236 | 42.90 | 50.30 | 33.60 | 209.64 | 188.05 | 25.90 | 2.25 | 162.15 | 16.85 | 0.10 | 25.00 | 1.50 | 234.65 | 158.70 | 263.75 | 72.79 | 27.21 | 86.90 | 96.30 | 9.30 | 27.56 | 235.11 | 11.72 |
| 92 | IP 291 | 43.00 | 52.20 | 31.40 | 209.71 | 248.45 | 27.80 | 2.55 | 220.65 | 15.85 | 0.07 | 25.85 | 1.50 | 113.65 | 90.85 | 166.00 | 32.42 | 67.58 | 82.30 | 95.50 | 8.98 | 26.59 | 134.59 | 19.81 |
| 93 | IP 230 | 44.20 | 53.10 | 32.50 | 262.46 | 204.55 | 19.25 | 2.15 | 185.30 | 18.60 | 0.10 | 28.25 | 1.60 | 183.50 | 108.80 | 227.05 | 64.21 | 35.79 | 83.10 | 97.00 | 11.80 | 34.96 | 161.19 | 21.72 |
| 94 | IP 262 | 42.50 | 50.00 | 33.45 | 273.10 | 236.70 | 22.55 | 2.60 | 214.15 | 17.20 | 0.08 | 24.10 | 1.50 | 179.65 | 101.65 | 223.65 | 65.47 | 34.53 | 86.70 | 94.10 | 9.83 | 29.11 | 150.59 | 19.36 |
| 95 | IP 231 | 43.50 | 51.90 | 33.45 | 289.82 | 235.80 | 18.90 | 2.75 | 216.90 | 16.65 | 0.08 | 20.90 | 1.60 | 146.85 | 110.30 | 191.05 | 45.18 | 54.82 | 84.30 | 95.80 | 11.50 | 34.07 | 163.41 | 20.94 |
| 96 | THAK 1827 | 42.70 | 53.20 | 33.30 | 274.83 | 228.60 | 24.95 | 2.80 | 203.65 | 20.95 | 0.10 | 24.70 | 1.50 | 249.15 | 162.65 | 325.85 | 54.53 | 45.47 | 86.90 | 97.50 | 13.35 | 39.56 | 240.96 | 16.43 |
| Mean | | 42.96 | 50.85 | 33.65 | 251.87 | 297.60 | 25.70 | 2.73 | 211.64 | 17.82 | .10 | 23.68 | 1.55 | 208.43 | 129.41 | 257.77 | 61.06 | 38.93 | 83.68 | 94.58 | 11..03 | 32.65 | 184.35 | 19.45 |
| Range | | 41.10-45.50 | 47.0-56.10 | 31.4-37.20 | 142.10-416.40 | 188.10-295.60 | 16.40-40.90 | 2.20-3.70 | 162.20-273.50 | 15.10-24.50 | 0.10 | 17.10-30.10 | 1.20-2.00 | 112.40-372.50 | 55.10-269.30 | 146.20-422.70 | 28.80-86.50 | 13.50-71.20 | 80.80-88.40 | 93.70-97..0 | 8.80-13.40 | 25.90-39.60 | 81.00-310.60 | 10.80-38.90 |
| SD | | 1.077 | 1.69 | 1.24 | 56.66 | 20.47 | 4.81 | 0.33 | 19.94 | 2.19 | 0.02 | 2.93 | 0.17 | 57.15 | 43.42 | 62,04 | 12.27 | 12.27 | 1.64 | 1.66 | 1.09 | 3.22 | 53.37 | 6.58 |

**Table 2. List of SSR markers used for screening of pearl millet genotypes**

| S. no. | SSR marker | Forward | Reverse |
|---|---|---|---|
| 1. | *Xibmsp01* | GCAGACTGAGAAGGCTTTCC | TGCTCTTCCAGAAGCGGTTG |
| 2. | *Xibmsp02* | GGAGTACAGAGTCCGCACATT | CTTCTCAACTTTGCGACAGGT |
| 3. | *Xibmsp03* | CGCAACAGAATTTTGTCGG | TTACGCTGGTTGTCAAGTTG |
| 4. | *Xibmsp04* | AGTGAGTCAAGATCTTCATTTTTCC | AAGGGAATGGCTTGAAGATT |
| 5. | *Xibmsp05* | TCTCCTTCTCCTTGCTGATGA | GCTGAAGTTGCAGCACAGAC |
| 6. | *Xibmsp06* | CGGTGCTCATGTACACATTC | TGATAGCCTGCTGCATGAAG |
| 7. | *Xibmsp07* | GTCCCTTGCGTGGAACAAAT | AGCTAAAGCCAGTTCCAGTG |
| 8. | *Xibmsp08* | ACTTGACTCCAACCTCCAAC | TGGGGATACAGATGCTGTAG |
| 9. | *Xibmsp09* | ATACGCCGAAGAGCTGTCAG | AGCGTAATGGCAGTCATGTC |
| 10. | *Xibmsp10* | GCTGGAGCTTGACTCGTG | CAAAGAGAAACGAAATTTCCACA |
| 11. | *Xibmsp11* | CGTCAATGGCATATCTACAC | CCATACCAATGTCATTGAGC |
| 12. | *Xibmsp12* | TTTTGTTATCCACAGTCCAACTC | TGCCTTAGAAGCATCTGCAA |
| 13. | *Xibmsp13* | GGAAGTCGTAGCAGAAGTTG | CAAGGTCTCCATCAACTGGC |
| 14. | *Xibmsp14* | TCTTCAGGGATGTTCCCTACT | GAGGAAGTTTATGATGGAAGGAAA |
| 15. | *Xibmsp15* | TGCTACGCCAATTTCTAATGC | CCACCATCGTCAAGTACTGC |
| 16. | *Xibmsp16* | GAGCTCCAGATGATGAACAC | CTTGCCATAGCACCAAATGG |
| 17. | *Xibmsp17* | *CATGGCACCACTAGACATAG* | *GAAACTGACTTCATGATGGAG* |
| 18. | *Xibmsp18* | ATAGATAAAACAGGTGCAGTTTCAGA | ATGACCACAGATCAGCCTTG |
| 19. | *Xibmsp19* | GTGTTGGTTCCATCTCAGG | CTGCCTCATGGTTATGATGG |
| 20. | *Xibmsp20* | GCTGAGCTTGACCTTGTTGTC | CCTGGCATGATTCCAATTTT |
| 21. | *Xibmsp21* | GAACCTCATCCAACAATTCC | GCTGCTGATGTTGCTATTGC |
| 22. | *Xibmsp22* | CGAATCCTCTTGGTACCAAC | GATCGCTCTTCATGTGGTTC |
| 23. | *Xibmsp23* | AAAGGACCAGTCACGTGAAG | ATAGCCTGGCCATTTCCTC |
| 24. | *Xibmsp24* | CATCATTGGCCCACACAAT | GAACAACTTAAGCTGGTAGATGC |
| 25. | *Xibmsp25* | GTGAAAAAGGGTCCAAAGGG | GAAGCCCCAGTAAGTCTTC |
| 26. | *Xibmsp26* | GAGGTTCGTCAAGAGGTTCG | TCCTCGGCCTCAATAAGCTA |
| 27. | *Xibmsp27* | CATTGCTCTTCATGGTGGAG | TGGAGCACTGAAGCCAGTAA |
| 28. | *Xibmsp28* | CGGCCGAGGTACTAACAGTC | GAGAAGCTAGGGGCAACCTT |
| 29. | *Xibmsp29* | GATGCAAATTTGTGGGAACC | GCCGAGACTCGAAAACAATC |
| 30. | *Xibmsp30* | AGACAGACAGCACGCACAAC | GAGCTCGACGACATGATGG |
| 31. | *Xibmsp31* | ATCGATCTTGTGTGCAGTGG | GACCCGACATGAGGACATTC |
| 32. | *Xibmsp32* | CTGGTGACCATGTCCTTCCT | TTGGTGGTTTGGCAACATTA |
| 33. | *Xibmsp33* | GAAGGAGAAGCACCACAAGC | CCGAGGATATCCAGATCGAA |
| 34. | *Xibmsp34* | GCTCGAAACACGAAACCCTA | CTGGCAGGTGACTTCTCCA |
| 35. | *Xibmsp35* | ACGAGATGTTCCTCGTCCTG | CCTCCTTGTTCGAGATGGTG |

### 3.1.4 Root-Shoot Ratio (R/S Ratio)

Root/shoot ratio varied in range of 0.06 cm to 0.12 cm with an average of 0.08. Maximum root/ shoot ratio was investigated with genotype IP177 (0.12 cm) intimately chased by a two genotypes *namely*: IP164 (0.115 cm) and IP154 (0.114).However, the lowest value was documented for the genotype IP180 (0.06).

### 3.1.5 Spike length (cm)

The mean values for panicle length ranged from 16.40 to 40.90 cm with the average mean of 25.68 cm. Genotype IP115 (40.90 cm) exhibited the highest panicle length followed by genotypes IP170 (38.70 cm) and IP174 (34.80 cm), whereas the minimum grain spike length was noted for the genotype IP159 (16.40).

### 3.1.6 Spike girth (cm)

The mean values for spike girth ranged between 2.15 cm to 3.75 cm with a grand mean of 2.71 cm. Genotype, IP198 (3.70) has highest spike girth followed by IP149 (3.60) and IP173 (3.45). However, the minimum was 2.15cm for genotype IP230.

### 3.1.7 Fresh weight (g)

Fresh weight ranged from 112.35 g to 372.45 g with a grand mean of 208.41 g. Maximum fresh weight value in grams was observed in genotype

IP104 (372.45 g) tracked by genotypes IP198 (369.50 g) and IP155 (332.65 g). While the lowest fresh weight value was recorded for the genotype IP153 (112.35 g).

### 3.1.8 Turgid weight (g)

Turgid weight varied from 146.15 g to 422.65 g with an average of 257.75 g. The maximum turgid weight value was recorded for genotype IP104 (422.65 g) tracked by two genotypes *viz.,* IP198 (409.80 g) and IP119 (408.75 g). However, the lowest turgid weight was observed for the genotype IP153 (146.15 g).

### 3.1.9 Dry weight (g)

Dry weight varied in range of 55.10 g to 269.25 g with an average of 129.39, maximum for the genotype IP155 (269.25 g) chased by genotypes IP104 (234.25 g) and IP180 (224.85 g). However the lowest worth was evidenced for the genotype IP 143(55.10 g).

### 3.1.10 Relative water content (RWC %)

RWC is considered as a prominent physiological parameter to predict tolerance against drought stress. RWC value of pearl millet genotypes varied in range of 28.80% to 86.47% with mean of 61.07%. Maximum RWC value was recorded with genotype IP188 (86.47%) closely chased by two genotypes IP195 (82.45%) and IP126 (80.45%). While the genotype IP119 (28.80%) was proved lowest performer in this regard.

### 3.1.11 Saturation water deficit (SWD %)

SWD value differed in range of 13.53% to 71.20% with an average of 38.93%. Minimum SWD was evidenced for the genotype IP188 (13.53%) intimately tracked by a group of two genotypes including IP195 (17.55%) and IP126 (19.55%). The highest SWD value was shown by genotype IP119 (71.20%).

### 3.1.12 Canopy temperature (°C)

Canopy temperature ranged between 31.40ºC to 37.15ºC with an average worth of 33.63ºC. Maximum canopy temperature value was witnessed for the genotype IP130 (37.15ºC) intimately tracked by two genotypes *viz*., IP183 (36.95ºC) and IP170 (36.10ºC), while the lowest canopy temperature was recorded for the genotype IP291 (31.40ºC).

### 3.1.13 Leaf area (cm sq/plant)

Leaf area varied in range of 142.07 $cm^2$ to 416.38 $cm^2$ with mean of 251.89 $cm^2$, maximum with genotype IP182 (416.38 cm sq/plant) intimately chased by genotypes IP192 (395.73cm sq/plant) and IP129 (382.95 cm sq/plant).While, the lowest Leaf area was covered by genotype IP 119 (142.07 cm sq/plant).

### 3.1.14 Days to 50 % flowering

Days to 50% flowering varied significantly in range of 47.00-56.10 days with an average of 50.86. Maximum numbers of days were taken to initiate 50% flowering by genotypes IP170 (56.10 days), IP115 (54.10 days) and IP154 (53.90 days).While the minimum days to initiate 50% flowering were taken by genotype IP198 (47.00 days).

### 3.1.15 Seed density of spike ($cm^2$)

Seed density varied significantly among ninety-six different pearl millet genotypes in range of 17.10-30.05 with an average mean of 23.66. Maximum Seed density was documented for the genotype IP120 (30.05) tracked by genotypes: IP180 (29.80) and IP164 (29.70). While minimum seed density (17.10) was documented for the genotype IP175.

### 3.1.16 Numbers of tillers per plant

The mean value of numbers of tillers ranged from 1.20 to 2.00 with the mean of 1.56. The maximum numbers of tillers was depicted by the genotype IP154 (2.00 tiller) tracked by genotypes IP155 (2.00 tillers), IP175 (1.9) and IP149 (1.90) whereas, the tillers in minimum numbers was observed with genotype IP109 (1.20).

### 3.1.17 Days to physiological maturity

Days of physiological maturity ranged from 80.80 days to 88.40 days with mean of 83.68 days. Maximum days to physiological maturity was taken by the genotype IP109 (88.40) intimately pursued by genotypes IP105 (87.70) and IP 117 (87.40). However, the lowest days to physiological maturity was recorded for the genotype IP169 (80.80).

**Table 3a. Correlation coefficient among different morpho-physiological traits of pearl millet germplasm lines**

| Traits | DAS_50 | DAF | CT | LA | PH | SL | SG | SHL | RL | SDS | NT |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DAS_50 | 1 | 0.459** | .095 | -0.61 | -0.162 | -0.27 | -0.174 | -0.160 | -0.64 | -0.164 | 0.110 |
| DAF | | 1 | .007 | -0.004 | -.156 | .170 | -0.176 | -0.201* | 0.004 | -0.03 | 0.235* |
| CT | | | 1 | -.112 | .076 | .144 | .027 | .043 | .063 | .062 | -.136 |
| LA | | | | 1 | .122 | .018 | .249* | .121 | -.071 | .088 | .118 |
| PH | | | | | 1 | .225* | .157 | .972** | -.018 | -.004 | -.031 |
| SL | | | | | | 1 | .132 | -.010 | -.140 | -.087 | .055 |
| SG | | | | | | | 1 | .129 | .051 | -.005 | .014 |
| SHL | | | | | | | | 1 | .015 | .017 | -.045 |
| RL | | | | | | | | | 1 | -.081 | .127 |
| SDS | | | | | | | | | | 1 | -.095 |
| NT | | | | | | | | | | | 1 |

**. Correlation is significant at the 0.05 level (2-tailed).*

***. Correlation is significant at the 0.01 level (2-tailed).*

*DAS_50= days of 50% heading initiation, DAF=Days of 50% flowering initiation, CP= Canopy Temp., LA= Leaf Area, PH= Plant Height, SL=Spike length, SG= Spike Girth, SHL= Shoot Length, RL=Root Length, SDS= Seed Density, NT= Number of Tiller*

**Table 3b. Correlation coefficient among different morpho-physiological traits of pearl millet germplasm lines**

| Traits | FW | DW | TW | RWC | SWD | DPM | DM | PPH | TSW | YLD | BYD | HI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FW | 1 | .859** | .942** | .410** | -.410** | .053 | .053 | .129 | .032 | .030 | .806** | -.700** |
| DW | | 1 | .856** | .001 | -.001 | .018 | .018 | .232* | -.073 | -.075 | .900** | -.835** |
| TW | | | 1 | .156 | -.156 | -.002 | -.002 | .134 | .039 | .036 | .799** | -.710** |
| RWC | | | | 1 | -1.000** | .134 | .134 | -.085 | .127 | .130 | .042 | .059 |
| SWD | | | | | 1 | -.134 | -.134 | .085 | -.127 | -.130 | -.042 | -.059 |
| DPM | | | | | | 1 | 1.000** | .013 | .063 | .063 | .031 | -.006 |
| DM | | | | | | | 1 | .013 | .063 | .063 | .031 | -.006 |
| PPH | | | | | | | | 1 | -.012 | -.015 | .236* | -.217* |
| TSW | | | | | | | | | 1 | 1.000** | .045 | .271** |
| YLD | | | | | | | | | | 1 | .045 | .274** |
| BYD | | | | | | | | | | | 1 | -.893** |
| HI | | | | | | | | | | | | 1 |

**. Correlation is significant at the 0.05 level (2-tailed).*

***. Correlation is significant at the 0.01 level (2-tailed).*

*FW= Fresh Weight, DW= Dry Weight, TW=Turgid Weight, RWC= Relative Water Content, SWD= Saturation Water Deficit, DPM= Days to Physiological Maturity, DM=Days to Maturity, TSW= Test Weight, YLD=Yield, BYD= Biological Yield, HI=harvest Index*

**Table 4. Locus specific SSR markers presenting major allele frequency, number of alleles, gene diversity and Polymorphic Information Content (PIC)**

| Marker | Major Allele frequency | Gene diversity | PIC value |
|---|---|---|---|
| Xibmsp01 | 0.9479 | 0.0987 | 0.0939 |
| Xibmsp03 | 0.8333 | 0.2778 | 0.2392 |
| Xibmsp06 | 0.8438 | 0.2637 | 0.2289 |
| Xibmsp07 | 0.8750 | 0.2188 | 0.1948 |
| Xibmsp09 | 0.8333 | 0.2778 | 0.2392 |
| Xibmsp26 | 0.7604 | 0.3644 | 0.2980 |
| Xibmsp29 | 0.7604 | 0.3644 | 0.2980 |
| Mean | 0.8363 | 0.2665 | 0.2274 |

**Table 5. Cluster of germplasm lines based on molecular data using UPGMA**

| Cluster | Number of germplasm lines | Name of germplasm lines |
|---|---|---|
| 1 | 1 | IP274 |
| 2 | 5 | IP132, IP156, IP190, IP160 and IP164 |
| 3 | 4 | IP177, IP172, IP116 and IP182 |
| 4 | 1 | IP 101 |
| 5 | 17 | IP138, IP137, IP159, IP187, IP262, IP108, IP130, IP175, IP179, IP192, THAK1827, IP118, IP139, IP114, IP152, IP171 and IP231 |
| 6 | 12 | IP165, IP189, IP110, IP183, IP104, IP140, IP111, IP112, IP131, IP141, IP158 andIP198 |
| 7 | 4 | IP115, IP122, IP134 and IP146 |
| 8 | 34 | IP127, IP128, IP129, IP133, IP135, IP142, IP143, IP145, IP147, IP149, IP153, IP154, IP155, IP161, IP162, IP166, IP168, IP169, IP173, IP174, IP178, IP180, IP181, IP185, IP186, IP188, IP194, IP195, IP196, IP291, IP108, IP109, IP123 and IP126 |
| 9 | 9 | IP167, IP107, IP119, IP120, IP150, IP170, IP230, IP236 and IP283 |
| 10 | 9 | IP136, IP199, IP117, IP105, IP163, IP121, IP144, IP151 and IP193 |

#### 3.1.18 Yield ($qth^{-1}$)

Yield is a complex character governed by polygenes and environmental factors and their interaction. Grain yield value per hectare varied significantly in range of 25.93-39.56$qtha^{-1}$ with an average value 32.65 $qtha^{-1}$. Whereas, the maximum grain yield value per plant was documented with the genotypes THAK1827 (39.56 $qtha^{-1}$) chased by IP156 (39.33 $qtha^{-1}$) and IP109 (39.19 $qtha^{-1}$).While the minimum grain yield value per plant was noted with genotype IP144 (25.93 $qtha^{-1}$).

#### 3.1.19 Biological yield ($qth^{-1}$)

Biological yield varied significantly in range of 81.04-310.59 with an average value of 184.35qt.Maximum biological yield value was documented with genotype IP198 (310.59$qtha^{-1}$) tracked by genotypes IP188 (287.18 $qtha^{-1}$) and IP134 (281.85 $qtha^{-1}$).While the minimum biological yield value was recorded with genotype IP143 (81.04 $qtha^{-1}$).

#### 3.1.20 Harvest index (%)

The harvest index ranged between 10.84 to 38.87 per cent with the mean of 19.45%. The maximum harvest index was depicted by the genotype IP143 (38.87 per cent) trailed by IP192 (36.20 per cent) and IP153 (35.78 per cent). However, minimum harvest index evidenced with the genotype IP145 (10.84%).

### 3.2 Analysis of Correlations between Morpho-physiological Traits

Days to 50% heading initiation showed a highly significant ($p < 0.01$) and negative correlation with days to 50% flower initiation (r= -0.459). Days to 50% flower initiation negatively and significantly correlated with shoot length (r= -0.201) and had positively significant correlation

with numbers of tiller (r= 0.235) at 5% significance level. Leaf area is positively and significantly correlated with spike girth (r= 0.249) at 5% level of significance. Plant height is highly, positively and significantly correlated with shoot length (r= 0.972) at 1% significance level. While It had positively and significantly correlated with spike length (r= 0.225). Fresh weight is positively and significantly correlated with dry weight (r= 0.859), turgid weight (r= 0.942) and biological yield (r= 0.806) at 1% significance level and highly and negatively correlated with saturation water deficit (r=0.410) and harvest index (r= -0.700) at 1% level of significance. Dry weight is highly and significantly correlated with turgid weight (r= 0.856), biological yield (r=0.900) and harvest index (r= 0.835) at 1% significance level and plant population at harvesting (r= 0.232) at 5% significance level. Whereas turgid weight is negatively and significantly correlated with harvest index (r= -0.710) and positively and significantly correlated with biological yield (r= 0.799) at 1% significance level. While relative water content is highly, negatively and significantly correlated with saturation water deficit (r= -1.000) at 1% significance level. Days of physiological maturity is positively and significantly correlated with days of maturity (r= 1.000) at 1% significance level. Test weight is positively and significantly correlated with yield (r= 1.000) and harvest index (r= 0.271) at 1% level of significance. However, yield had positive and significant correlation with harvest index (r= 0.274) at 1% significance level. While biological yield is negatively and significantly correlated with harvest index (r= - 0.893) at 1% significance level (Table 3a & b).

### 3.3 Cluster Analysis of Morpho-physiological Traits

Cluster analysis of morpho-physiological traits was done on the basis of Jaccard's similarity coefficient using NTSYS ver 2.0 software. Dendrogram formed two clusters one major and one minor (Fig. 1). Minor cluster had one genotype *i.e.*, IP104 and major cluster had 95 germplasm lines which further divided in two groups one minor cluster and one major cluster. Minor cluster consist 16 germplasm lines *namely*; THAK1827, IP107, IP231, IP140, IP291, IP139, IP283, IP236, IP274, IP196, IP146, IP230, IP163, IP198, IP147 and IP114. Major cluster had 83 germplasm lines and it further divided in to two groups one major and one minor. Minor cluster contain 21 germplasm lines including IP193, IP188, IP105, IP101, IP119, IP167, IP154, IP 129, IP156, IP122, IP152, IP165, IP153, IP143, IP141, IP149, IP109, IP108, IP120, IP169 and IP183. Major cluster consist 61 germplasm lines which again divided into two clusters one minor and one major. Minor cluster had 22 germplasm lines (Fig. 1).

### 3.4 Principal Component Analysis (PCA)

Principal component analysis (PCA) was done by considering all the 23 morpho-physiological variables concurrently. The pattern of variations illustrated by the PCA described by correlation coefficients determined for pair-wise association of different morpho-physiolgical characters. The PCA correlation depicted that the accessions acquired higher values are occupying unique position towards the right side of the graph. Genotypes *viz*., IP198, IP182, IP119, IP194, IP192 and IP179 possess unique position on the plot and highest variance was showed at 65% on the basis of their phenotypic characters (Fig. 2).

### 3.5 Molecular Analysis

Among 35 SSR markers used in the current experiment, twenty-two were successfully amplified across all genotypes. Out of these 22 SSR markers, fifteen were monomorphic however, seven were found to be polymorphic across 96 germplasm lines of pearl millet (Table 4). The range of major allele frequency value was 0.7604 to 0.9479 with the average 0.8363. The highest major allele frequency value (0.9479) was observed for the markers Xibmsp01 chased by Xibmsp07 (0.8750), Xibmsp06 (0.8438), Xibmsp03 (0.8333) and Xibmsp09 (0.8333) while the lowest (0.7604) for the markers Xibmsp26 and Xibmsp29. The range of genetic diversity value was 0.0987 to 0.3644 with an average of 0.2665. The highest genetic diversity value (0.3644) was demonstrated by markers Xibmsp26 and Xibmsp29 trailed by Xibmsp03 (0.2778), Xibmsp29 (0.2778), Xibmsp06 (0.2637) and Xibmsp07 (0.2188) while the lowest (0.0987) was in marker Xibmsp01. The range of PIC value was 0.0939 to 2980 with the average 0.2274. The highest PIC value was recorded for the markers Xibmsp26 and Xibmsp29 (0.2980) pursued by Xibmsp03 (0.2392), Xibmsp29 (0.2392), Xibmsp06 (0.2289) and Xibmsp07 (0.1948) whereas the lowest for the markers Xibmsp01 (0.0939).

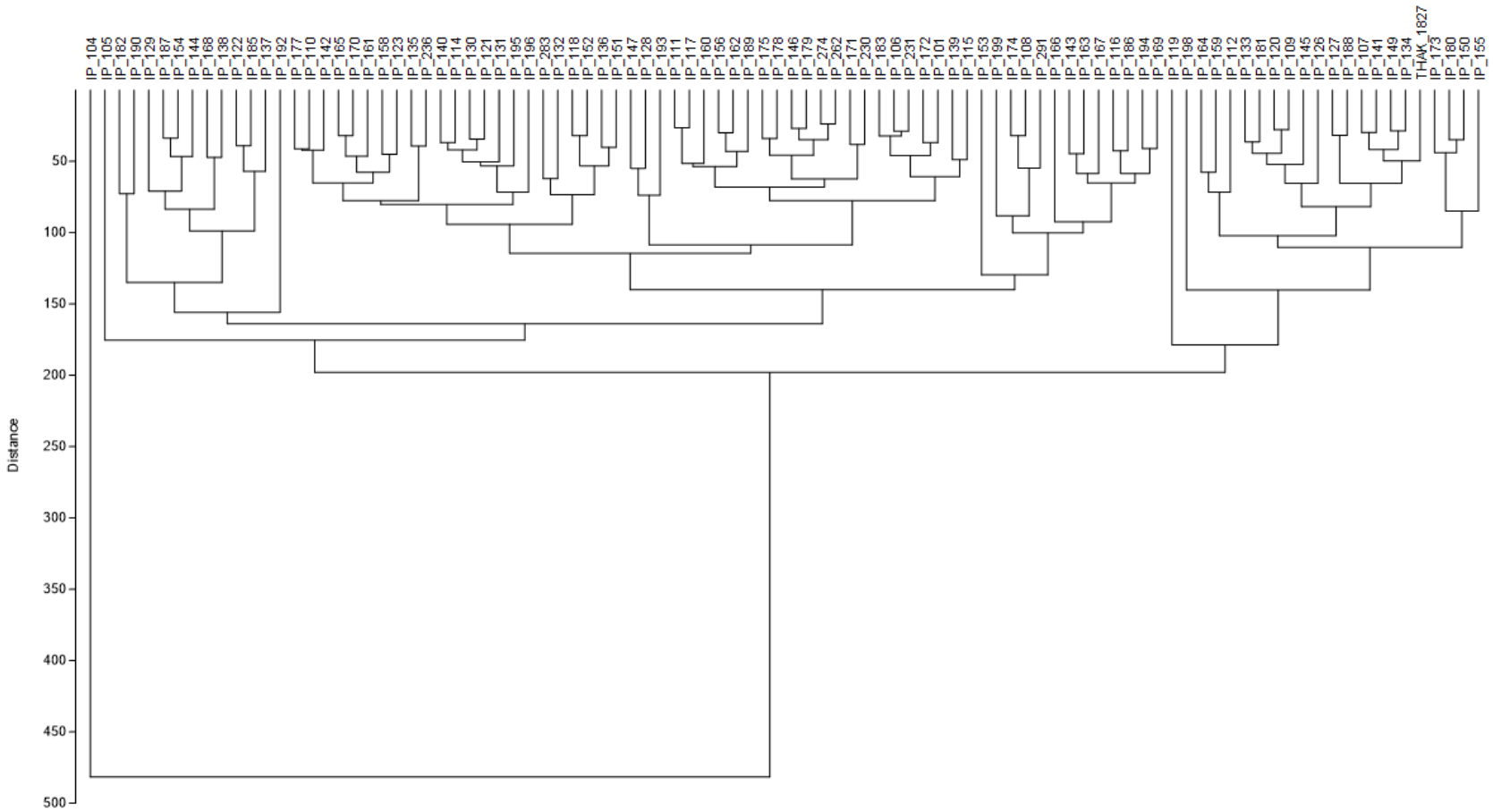


**Fig. 1. Dendrogram of pearl millet germplasm lines based on different morpho-physiological traits**

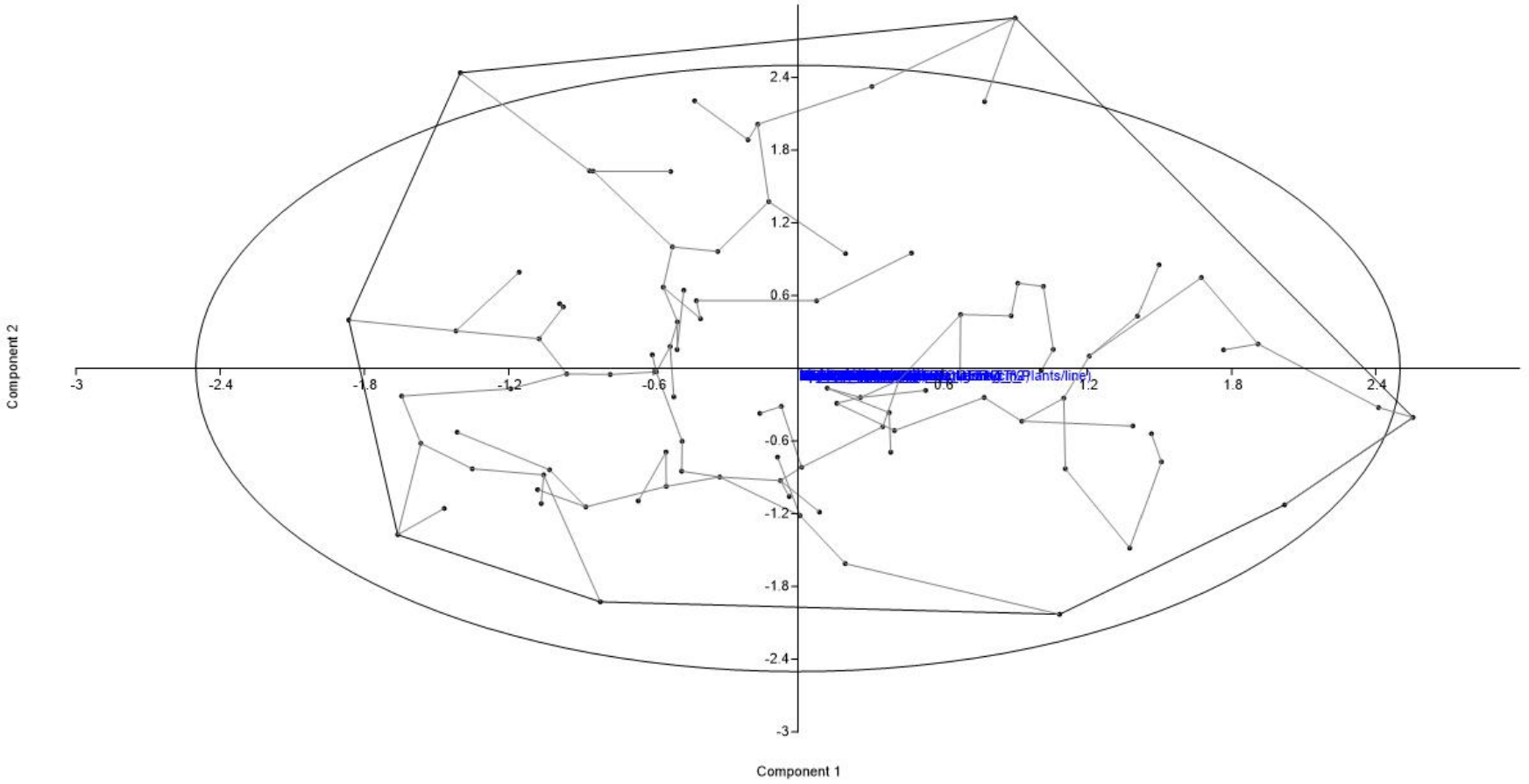


**Fig. 2. PCA diagram of pearl millet germplasm lines based on different morpho-physiological traits**

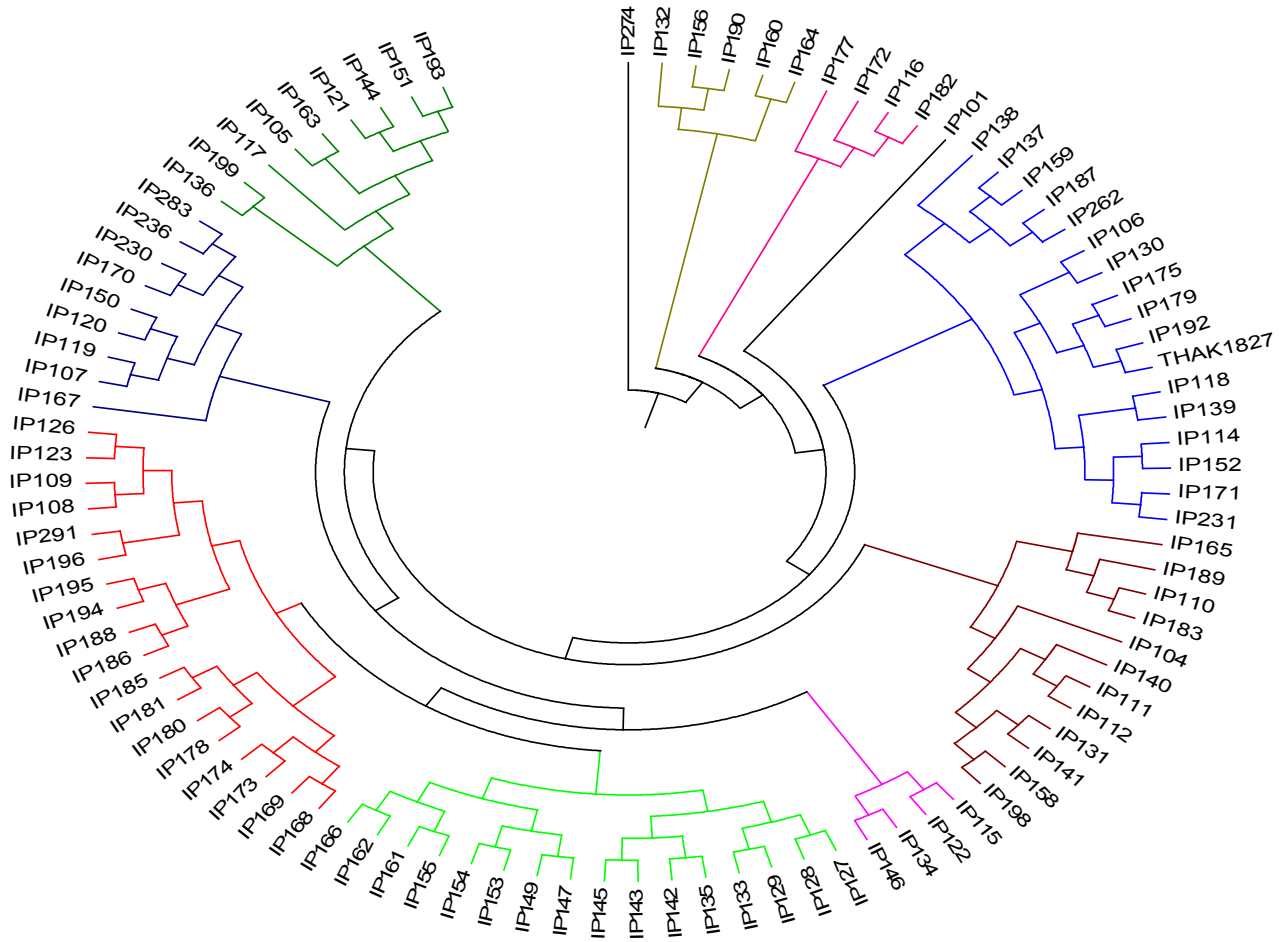


**Fig. 3. Dendrogram of 96 pearl millet germplasm lines showing clusters based on similarity using UPGMA relationship**

The genetic relationships among pearl millet germplasm lines are presented in UPGMA tree (Fig. 3). The clustering was based on genetic similarity between and among investigated pearl millet germplasm lines. Initially 96 pearl millet germplasm lines were divided into two clusters minor and major (Fig. 3; Table 5). Minor cluster contained one germplasm *i.e*., IP 274 (Highly diverse). The major cluster contained 95 germplasm lines and further divided into two clusters one major and one minor. Minor cluster had five germplasm lines. Among these five germplasm lines genotypes IP132, IP156 and IP190 showed high similarity and grouped together. In the same way, genotypes IP160 and IP164 also grouped together. The major cluster had 90 germplasm lines and it was further divided into two subclusters: minor and major subclusters. Minor subcluster contains 4 germplasm lines including IP177, IP172, IP116 and IP182. Major subcluster had 86 germplasm lines and further sub divided into two different major and minor groups.The minor cluster had only one germplasm *i.e*., IP101 and major had 85 germplasm lines (Fig. 3).

## 4. DISCUSSION

### 4.1 Morpho-physiological Variability

Drought is the most devastating abiotic constraint affecting crop productivity, which is caused by insufficient precipitation and/or altered rainfall patterns. Drought stress causes many different physiological reactions in plants. Drought, being the most important abiotic stress, severely impairs plant growth and development, limits plant production and the performance of crop plants, more than any other abiotic factor. Pearl millet is one of cereal which has strong development of roots and tends to have effective adaptive mechanism to cope with drought. Several morpho-physiological characters are determining processes in plants respond to drought stress. Drought effects growth, yield, membrane integrity, pigment content, osmotic adjustment, water relations and photosynthetic activity [26,27]. In present research, all 96 pearl millet germplasm lines differed significantly in plant height, days to 50% flowering, leaf area, fresh weight, dry weight, relative water content, days to physiological maturity, test weight, yield and biological yield. Correlation studies give a clear depiction of characters alliance which is generally as a result of linkage, pleiotrophy, physiological relationship in developmental pathway. The quantitative measurement of individual character gives an interpretation of different variability parameters.

Relative water content (RWC) and canopy temperature are important features that influence plant water relationships. RWC is related to water uptake by the roots as well as water loss by transpiration [28]. RWC was recorded highest in genotypes IP188 (86.47%) tracked by IP195 (82.45%) and IP 126 (80.45). Canopy temperature was recorded highest in genotypes IP130 (37.15$^{o}$C) trailed by IP183 (36.95ºC) and IP170 (36.10ºC). Genotypes with high percentage of RWC showed their tolerance against drought. Similar results were also obtained by Schonfeld et al. [29]. The observations on leaf area, days to 50% flowering and physiological maturity showed that the pearl millet germplasm lines took lesser number of days to reach maturity under drought condition. The drought stress resulted in early flowering and maturity as well as decrease in leaf growth. Leaf area was recorded highest in genotypes IP182 (416.38 cm sq/plant). Days to 50% flowering initiation was recorded highest in IP170 (56.10 days). Days of physiological maturity was recorded in range 80.80 days to 88.40 days with highest in genotype IP109 (88.40). Winkel et al. [30] had reported parallel results.

Yield is a complex characteristic that is governed by large number of genes, biotic and abiotic factors and their effects on plants. Drought stress leads to severe decline in yield. Spike length and seed weight are the important contributors of yield in pearls millet. Droughts bring lesser spike length and low-test weight resulting in decrease in yield. Similar findings have been reported earlier by Farooq et al. [31] and Anjum et al. [28].

### 4.2 Molecular Variability

The assessment of genetic variability owing to morpho-physiological features alone might not provide an accurate classification of the genetic divergence between the genetic resources, may be due to the environmental influence and development-specific trait appearance. The application of molecular markers provides a better assessment of genetic variability present in the breeding materials. In the present investigation with 96 germplasm lines to characterize the diversity at molecular level the 35 gene–linked SSR molecular markers were employed and presented appreciate information about genetic diversity existing in pearl millet germplasm lines. For effective genetic variability

analysis, allele frequency, genetic diversity and polymorphism information content for each SSR locus were assessed. However; the lower Polymorphic Information Content (PIC) values show low allelic diversity in current investigation of pearl millet germplasm lines. Kapila et al. [12] and Singh et al. [32] also reported similar findings. The SSR allelic variability detected among pearl millet genotype in this study was found to be low in comparison to earlier investigation [33].

Then dendrogram generated on the basis of SSR markers grouped most of the genotypes in the same cluster. This indicates low level of diversity present among the genotypes at molecular level. It could be related to seeds exchange among regions which highly contributed to inter mixture of accessions. However, this may also be due to the use of a small number of SSR markers in the present study. For accurate identification of genetic variability more numbers of markers should be used.

## 5. CONCLUSION

In conclusion, genotypes *viz*: IP133, IP127, IP177, IP198, IP107, IP140, IP164, IP181, IP160, IP166, IP194, IP195, IP126, IP190, IP196, IP158, IP178, IP121, IP110, IP236, IP230 and THAK 1827 made their position in distinct group regarding drought by using different morpho-physiological and SSR molecular markers. So these germplasm lines may be used as a donor parent for future breeding programme for development of drought tolerant genotype(s) identification of QTLs by development of RILs through forward genetics approaches.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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