



# Evaluating Pearl Millet Germplasm for Morphological and Biochemical Parameters

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## ABSTRACT

The study was conducted during the *kharif* season (July–October, 2024) at Rajmata Vijayaraje Scindia Krishi Vishwa Vidyalaya, Gwalior, Madhya Pradesh, to assess genetic diversity among thirty pearl millet (*Pennisetum glaucum* L.) germplasm lines based on morphological and biochemical traits. Total eleven morphological observations including grain yield, dry fodder yield, days to 50% flowering, days to maturity, plant height, number of productive tillers, panicle length, panicle diameter, 1000 seed weight, plant population and seed set under bagging and four biochemical parameters including total protein, total soluble sugar, total phenolic contents and DPPH were recorded. Grain yield was highly significant and positively correlated with dry fodder yield ( $r=0.67$ ), panicle diameter ( $r=0.50$ ), and plant population at harvest ( $r=0.50$ ) at 1% significant level. Morphological observations showed diversity among the pearl millet germplasm. Similarly, there was variation between biochemical observations i.e., protein content varied between 9.2–11.5%, total soluble sugars between 1.4–2.6 g 100 g<sup>-1</sup>, phenolic content between 20.14–34.78 and DPPH scavenging activity varied between 44.18–54.89%. Cluster analysis revealed two major groups, and variability between the expression pattern of these observations. Biochemical profiling supported by heatmap analysis showed considerable diversity, with HHB 299 and Dhanshakti displaying superior protein content, total phenolics, and antioxidant activity, emphasizing their nutritional advantage. The findings emphasize the importance of integrating morphological and biochemical profiling in breeding programs to develop high-yielding, nutrient-rich, and climate-resilient pearl millet cultivars, vital for enhancing food security and public health under challenging agro-climatic conditions.

**KEYWORDS:** Pearl millet, morphological, biochemical parameters, antioxidant activity

**Citation (VANCOUVER):** Rathore et al., Evaluating Pearl Millet Germplasm for Morphological and Biochemical Parameters. *International Journal of Bio-resource and Stress Management*, 2025; 16(8), 01-10. [HTTPS://DOI.ORG/10.23910/1.2025.5862](https://doi.org/10.23910/1.2025.5862).

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**Data Availability Statement:** Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

**Funding:** The research was conducted with the kind supports from Indian Council of Agricultural Research, National Agricultural Science Fund (NASF), New Delhi and AICRP Pearl millet

**Conflict of interests:** The authors have declared that no conflict of interest exists.

## 1. INTRODUCTION

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) is a vital grain crop cultivated extensively in arid and semi-arid regions, renowned for its remarkable resilience to harsh environmental conditions. Native to the African Sahel, it has spread globally and is predominantly grown in Asia and Africa. Its resistance to drought, high temperatures, and low soil fertility makes it indispensable in areas where crops like wheat, rice, and maize fail. India, the leading producer of pearl millet in Asia, heavily relies on this crop, especially in its arid and rainfed agricultural zones. India cultivates over 6.70 mha of pearl millet, producing 9.62 mt with an average productivity of 1436 kg ha<sup>-1</sup> (Anonymous, 2022). Since the 1980s, the cultivated area has decreased by 22%, but output has risen by 36%, driven by a 75% increase in productivity—from 530 kg ha<sup>-1</sup> (1981–1983) to 941 kg ha<sup>-1</sup> (2009–2011) (Yang et al., 2024). Pearl millet's adaptability to extreme conditions—tolerating drought and temperatures up to 45°C—establishes it as a cornerstone for climate-resilient agriculture (Satyavathi et al., 2021; Bheemaiah et al., 2024). Climate change intensifies stresses like prolonged droughts, rising temperatures, and unpredictable rainfall, which threaten agricultural productivity (Jukanti et al., 2017; Saleem et al., 2024). Its deep root system, efficient water utilization, and ability to thrive in marginal soils ensure sustained productivity in challenging environments. Consequently, pearl millet plays a crucial role in safeguarding food security and supporting the livelihoods of smallholder farmers. Beyond its resilience, pearl millet is celebrated for its rich nutritional profile, surpassing other cereals like rice, sorghum, and wheat. A 100 g serving provides 360 calories, 12 g protein, 5 g fat, 2 g minerals, 1 g fiber, 67 g carbohydrates, 42 mg calcium, and 242 mg phosphorus (Satyavathi et al., 2021). Its high levels of iron and zinc help combat micronutrient deficiencies, often referred to as "hidden hunger," which is widespread in rural and underprivileged areas of India (Lowe et al., 2021; Onyeje et al., 2022). Moreover, its elevated calcium content contributes to improved bone health, particularly in children and the elderly. Gluten-free by nature, pearl millet is suitable for individuals with celiac disease or gluten sensitivities (King et al., 2019; Anitha et al., 2021). Its low glycemic index supports diabetes management, providing a healthier alternative to refined cereals. The crop's versatility extends to its use in value-added products, including cakes, biscuits, pasta, and parboiled substitutes for rice (Schober et al., 2005). Enhanced nutritional qualities can be leveraged through breeding programs to develop varieties suitable for both human consumption and animal feed (Kumar et al., 2022). Such initiatives align with global efforts to address malnutrition and ensure sustainable food systems. With increasing environmental and nutritional challenges, the demand for improved pearl millet germplasm is growing.

Breeding efforts aim to enhance agronomic traits and nutritional profiles, creating robust cultivars that thrive in harsh climates while addressing dietary needs (Gautam et al., 2024; Sharma et al., 2024). Morphological and genetic evaluations of diverse germplasm play a key role in identifying superior lines for breeding programs. In India, where rainfed agriculture supports a significant portion of the population, these advancements are crucial for improving yields and ensuring food security (Rao et al., 2015; Sarkar et al., 2020). This study focuses on assessing the morphological traits and nutritional value of pearl millet germplasm, aiming to develop climate-resilient and nutritionally enriched varieties. This research contributes to sustainable agriculture, enhances farm productivity, and addresses malnutrition. Pearl millet's intrinsic resilience and nutritional value position it as a vital crop for combating the dual challenges of climate change and food insecurity and public health in vulnerable regions.

## 2. MATERIALS AND METHODS

A laboratory experiment was carried out during the *Akharif* (July–October, 2024) at the Department of Molecular Biology & Biotechnology, College of Agriculture, Rajmata Vijayaraje Scindia Agricultural University, Gwalior, Madhya Pradesh, India (Lat 26.220 and Long 78.199). The field experiment was conducted at Research Farm, Rajmata Vijayaraje Scindia Krishi Vishwavidyalaya, Gwalior, Madhya Pradesh, India.

### 2.1. Plant material

Thirty distinct pearl millet germplasm, comprising both commercial cultivars and newly developed hybrids, were cultivated during the Kharif season of July–October 2024. Each genotype was planted in a single row, 3 meters in length, with a plant-to-plant spacing of 10 cm. The germplasm was arranged in a randomized block design. Throughout the growing season, standard cultural practices were followed, including the recommended fertilization of 20 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as a baseline and 10 kg N ha<sup>-1</sup>. Observations were recorded based on individual plants, selecting three randomly chosen plants replication for each characteristic.

### 2.2. Morphological observations

Morphological data was taken for grain yield (kg net plot<sup>-1</sup>), dry fodder yield (kg net plot<sup>-1</sup>), days to 50% flowering, days to maturity, plant height (cm.), productive tillers (No plant<sup>-1</sup>), panicle length (cm.), panicle diameter (cm.), 1000-Seed wt.(g), population at harvest\_no. net plot<sup>-1</sup>, seed set under bagging (%). Similar parameters have been considered Makwana et al., 2023 for evaluating different genotypes for morpho-physiological traits at field conditions.

### 2.3. Biochemical observations

Prior to biochemical examination, Pearl millet grains

were manually purified to eliminate contaminants and subsequently pounded into a fine powder utilising a mortar and pestle. The powdered grains were defatted using acetone at a 1:10 weight-to-volume ratio for 24 hours to remove lipids. The defatted grains were subjected to filtration and subsequently dried in a hot air oven at 80°C for 24 hours to remove residual acetone and moisture. Ultimately, 10 g of the desiccated grains were manually pulverised for ensuing biochemical investigation. Total soluble proteins were quantified utilising the method established by Lowry et al. (1951). 100 mg of grain powder was homogenised in ten millilitres of 50 mM cold potassium phosphate buffer (pH 7.0) and subsequently centrifuged at 15,000×g for 20 minutes at 4°C. The supernatant was filtered and preserved at 0°C. For protein quantification, 0.1 ml of extract was combined with 2.9 ml of reagent A and 1 ml of 1N Folin-Ciocalteu reagent, incubated in the dark for 30 minutes, and subsequently detected at 660 nm. Protein content was quantified as mg protein gramme<sup>-1</sup> of fresh weight. The total phenolic content in pearl millet was quantified using the Folin-Ciocalteu colorimetric method (Singleton et al., 1999). The reaction mixture consisted of a methanolic solution, extract, distilled water, sodium carbonate, and Folin-Ciocalteu reagent, which was let to stand for 2 hours in darkness. Absorbance was measured at 725 nm. Phenol concentrations were determined using the calibration curve and represented as mg of catechol equivalents of phenol gramme<sup>-1</sup> of fresh fruit weight (FW). Samples beyond 800 µg ml<sup>-1</sup> were diluted prior to analysis. The DPPH radical concentration was assessed using the methodology outlined by Lo Scalzo et al. (2004). The impact of ethanolic extracts on DPPH radical levels with minor alterations. A 0.5 ml aliquot of 0.48 mM DPPH solution was combined with 1.0 ml of methanol and 1.0 ml of ethanolic extracts at different concentrations. The solution was agitated and incubated in darkness for 90 minutes. Absorbance was quantified at 517 nm at ambient temperature, with all measurements performed in triplicate. EC50 values were derived using a calibration curve utilising quantity of ethanolic extracts (Sanchez-Moreno et al., 1998). Total soluble sugars (TSS) were quantified according to the modified technique of Hansen and Moller (1975). Two hundred microlitres of extract were evaporated in triplicate using a 100°C water bath. Subsequent to evaporation, 1 ml of double-distilled water was introduced and vortexed. A blank was produced using 1 ml of water in each tube. D-glucose concentrations ranging from 0.01 to 0.1 mg were created in individual tubes, each adjusted to a final volume of 1 ml. Subsequently, 4 ml of ice-cold anthrone reagent was introduced to each tube, which were then incubated at 80°C for 8 minutes. The reaction produced a green-blue hue, which was quantified spectrophotometrically at 630 nm. TSS was measured in

grammes 100 grammes<sup>-1</sup> of dry weight and determined using a d-glucose standard curve. All absorbance measurements were conducted using a UV-visible spectrophotometer (UVD 3200, Labomed, INC).

#### 2.4. Statistical analysis

The correlation coefficient for all morphological characteristics was computed using SPSS version 19 software. Similarity matrices were utilised to create a dendrogram for all pearl millet germplasm lines using NTSYS-pc V 2.0 (Rohlf et al., 2000) based on the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The heat map and dendrogram analysis was conducted utilising the "heatmap" function from the 'R' package (Zhao et al., 2014).

### 3. RESULTS AND DISCUSSION

#### 3.1. Morphological statistical data assessment

The identification of pearl millet germplasm is determined through a set of physical traits. These include grain yield, which ranged from 2.14 to 4.442 kg net plot<sup>-1</sup>, and dry fodder yield, varying between 5.00 to 11.50 kg net plot<sup>-1</sup>. Additionally, days to 50% flowering ranged from 34 to 48 days, while days to maturity spanned 82 to 90 days. Other significant traits measured were plant height (177 to 280 cm), productive tillers (1 to 3), panicle length (18.9 to 35.2 cm), panicle diameter (1.2 to 3.2 cm), 1000 seed weight (8.2 to 12.4 g), and seed set under bagging, which varied from 80 to 100 seeds (Table 1). This data provides a comprehensive understanding of the key traits used to differentiate germplasm. Similar character was studied by Makwana et al., 2021 in pearl millet.

#### 3.2. Correlation coefficient analysis

Significant ( $p > 0.01$ ) and positive relationships were identified between grain yield and dry fodder yield ( $r = 0.67$ ), followed by population at harvest ( $r = 0.50$ ) and panicle diameter ( $r = 0.50$ ). Consequently, these characteristics may function as selection indices for enhancing grain production in pearl millet, as grain yield is a multifaceted trait influenced by several contributing factors as these traits increase, grain yield is also likely to improve (Table 2). Consequently, character association was examined in this study to evaluate the links among yield and its components to improve the efficacy of selection. The findings align with previous research on pearl millet conducted by various researchers, including Pareek (2002), Borkhataria et al. (2005), Izge et al. (2006), Kale et al. (2011), Atif et al. (2012), Angarawai et al. (2015), Kumar et al. (2016), Singh and Singh (2016) and Talawar et al. (2017), demonstrated a positive correlation of varying intensity among grain yield, plant population, and panicle diameter.

Table 1: Morphological observations of pearl millet germplasm for yield attributing traits

| Variety name       | Grain yield (kg/net plot) | Dry fodder yield (kg/net plot) | Days to 50% flowering | Days to maturity | Plant height (cm.) | Productive tillers (No./plant) | Panicle length (cm.) | Panicle diameter (cm.) | 1000-seed wt. (g) | Population at harvest/no. net plot | Seed set under bagging (%) |
|--------------------|---------------------------|--------------------------------|-----------------------|------------------|--------------------|--------------------------------|----------------------|------------------------|-------------------|------------------------------------|----------------------------|
| MPMH 35            | 3.29                      | 6.90                           | 36.67                 | 83.33            | 193.00             | 2.20                           | 21.00                | 2.00                   | 8.67              | 188.67                             | 86.67                      |
| RHB 223            | 3.30                      | 4.90                           | 37.33                 | 84.33            | 215.00             | 1.40                           | 20.37                | 2.60                   | 8.93              | 174.00                             | 90.00                      |
| PB1756             | 2.65                      | 5.47                           | 35.33                 | 86.00            | 215.00             | 1.47                           | 23.53                | 2.70                   | 10.13             | 166.33                             | 66.67                      |
| MPMH 21            | 2.45                      | 5.68                           | 37.33                 | 83.67            | 228.00             | 1.47                           | 23.93                | 2.80                   | 9.27              | 157.33                             | 78.33                      |
| GHB 719            | 3.29                      | 6.77                           | 39.67                 | 86.33            | 189.00             | 2.73                           | 19.27                | 2.27                   | 8.40              | 176.00                             | 90.00                      |
| HHB 67             | 2.23                      | 5.67                           | 35.33                 | 85.67            | 194.33             | 1.70                           | 20.47                | 2.57                   | 8.43              | 190.33                             | 100.00                     |
| Improved           |                           |                                |                       |                  |                    |                                |                      |                        |                   |                                    |                            |
| 86M94              | 4.44                      | 10.10                          | 30.33                 | 86.67            | 229.00             | 2.40                           | 22.80                | 2.93                   | 10.77             | 191.00                             | 81.67                      |
| DHBH 1397          | 4.52                      | 10.50                          | 40.67                 | 87.67            | 229.00             | 2.13                           | 24.57                | 3.13                   | 11.10             | 179.00                             | 75.00                      |
| JKBH1326           | 4.24                      | 9.50                           | 40.67                 | 87.33            | 217.67             | 1.97                           | 23.87                | 2.70                   | 9.60              | 171.67                             | 86.67                      |
| PB 1852            | 4.27                      | 9.60                           | 41.00                 | 85.67            | 214.33             | 1.80                           | 22.73                | 2.83                   | 9.20              | 158.00                             | 96.67                      |
| PB 1705            | 3.55                      | 6.67                           | 42.33                 | 87.67            | 207.00             | 2.40                           | 22.70                | 2.83                   | 10.03             | 195.33                             | 93.33                      |
| 86M01              | 4.42                      | 9.40                           | 42.00                 | 87.33            | 210.33             | 1.73                           | 25.20                | 2.73                   | 9.50              | 182.00                             | 86.67                      |
| MPMH 17            | 4.19                      | 8.30                           | 41.33                 | 87.00            | 212.33             | 2.13                           | 22.20                | 2.70                   | 9.33              | 159.00                             | 60.67                      |
| GHB 905            | 3.09                      | 6.42                           | 39.00                 | 82.67            | 177.33             | 1.67                           | 23.03                | 2.57                   | 8.93              | 175.00                             | 83.33                      |
| GHB 732            | 4.13                      | 10.77                          | 40.33                 | 86.67            | 193.00             | 2.13                           | 19.53                | 2.40                   | 8.33              | 182.00                             | 97.67                      |
| GHB 744            | 3.27                      | 6.17                           | 39.33                 | 88.00            | 212.00             | 1.47                           | 23.37                | 2.53                   | 8.87              | 158.00                             | 90.00                      |
| MP7878             | 3.70                      | 9.83                           | 45.67                 | 87.67            | 240.33             | 2.10                           | 25.90                | 3.07                   | 12.40             | 168.67                             | 81.67                      |
| 86M84              | 3.26                      | 10.30                          | 47.33                 | 88.67            | 241.33             | 1.47                           | 24.90                | 2.53                   | 11.10             | 175.33                             | 90.00                      |
| KBH 108            | 4.10                      | 10.30                          | 47.00                 | 89.00            | 252.00             | 1.30                           | 25.77                | 3.30                   | 10.47             | 150.00                             | 90.00                      |
| Kaveri super boss  | 3.33                      | 9.33                           | 46.33                 | 87.67            | 260.00             | 1.73                           | 28.97                | 2.80                   | 9.07              | 166.33                             | 100.00                     |
| 86 M 86            | 2.40                      | 7.07                           | 45.00                 | 88.67            | 229.33             | 1.47                           | 24.27                | 3.13                   | 11.93             | 128.00                             | 100.00                     |
| AHB 1269           | 2.22                      | 7.23                           | 40.33                 | 86.33            | 180.33             | 1.60                           | 22.10                | 2.83                   | 9.30              | 149.67                             | 83.33                      |
| AHB 1200           | 3.22                      | 6.87                           | 36.33                 | 86.00            | 204.67             | 1.93                           | 24.23                | 2.77                   | 9.93              | 138.00                             | 90.00                      |
| HHB 299            | 3.23                      | 7.33                           | 39.33                 | 87.00            | 191.00             | 1.13                           | 21.93                | 2.60                   | 8.23              | 134.00                             | 86.67                      |
| Dhanshakti         | 2.32                      | 6.33                           | 34.33                 | 84.33            | 217.00             | 2.03                           | 20.90                | 2.27                   | 10.00             | 135.00                             | 86.67                      |
| ICMV 221           | 2.29                      | 7.77                           | 39.33                 | 87.67            | 212.33             | 2.00                           | 22.13                | 2.60                   | 8.63              | 127.33                             | 93.33                      |
| Pusa composite 701 | 3.19                      | 11.00                          | 44.33                 | 88.33            | 280.00             | 2.47                           | 34.47                | 2.50                   | 8.93              | 140.00                             | 100.00                     |
| Pusa composite 383 | 3.15                      | 9.63                           | 41.67                 | 87.67            | 281.00             | 2.07                           | 34.90                | 1.93                   | 8.63              | 150.67                             | 85.00                      |
| JBV 2              | 2.65                      | 6.97                           | 44.67                 | 87.33            | 239.67             | 2.60                           | 30.07                | 1.47                   | 8.60              | 132.33                             | 100.00                     |
| RAJ 171            | 2.51                      | 6.13                           | 43.67                 | 87.67            | 206.67             | 2.00                           | 26.87                | 1.27                   | 8.93              | 117.00                             | 83.33                      |
| Mean               | 3.30                      | 7.96                           | 40.47                 | 86.67            | 219.07             | 1.89                           | 24.20                | 2.58                   | 9.52              | 160.53                             | 87.78                      |
| C.V.               | 4.57                      | 5.23                           | 7.37                  | 1.04             | 0.50               | 7.17                           | 2.09                 | 3.73                   | 3.99              | 3.07                               | 6.64                       |
| SEm±               | 0.09                      | 0.24                           | 2.42                  | 0.52             | 0.63               | 0.08                           | 0.29                 | 0.06                   | 0.22              | 2.85                               | 3.37                       |
| CD ( $p=0.05$ )    | 0.25                      | 0.68                           | 5.86                  | 1.48             | 1.79               | 0.22                           | 0.83                 | 0.16                   | 0.62              | 5.07                               | 5.53                       |

Table 2: Correlation coefficients among morphological traits in pearl millet germplasm

|     | GYD | DFY     | DF     | DM      | PH      | TN     | PL      | PD      | TSW     | PP      | SSB    |
|-----|-----|---------|--------|---------|---------|--------|---------|---------|---------|---------|--------|
| GYD | 1   | 0.670** | 0.106  | 0.219   | 0.123   | 0.184  | -0.021  | 0.369*  | 0.213   | 0.503** | -0.188 |
| DFY |     | 1       | 0.434* | 0.543** | 0.523** | 0.229  | 0.401*  | 0.289   | 0.293   | 0.151   | 0.105  |
| DF  |     |         | 1      | 0.650** | 0.465** | -0.048 | 0.516** | 0.010   | 0.225   | -0.248  | 0.290  |
| DM  |     |         |        | 1       | 0.519** | 0.056  | 0.458*  | 0.147   | 0.332   | -0.249  | 0.205  |
| PH  |     |         |        |         | 1       | 0.112  | 0.831** | 0.051   | 0.296   | -0.206  | 0.153  |
| TN  |     |         |        |         |         | 1      | 0.175   | -0.374* | -0.097  | 0.148   | 0.087  |
| PL  |     |         |        |         |         |        | 1       | -0.233  | 0.055   | -0.353  | 0.156  |
| PD  |     |         |        |         |         |        |         | 1       | 0.569** | 0.296   | -0.122 |
| TSW |     |         |        |         |         |        |         |         | 1       | 0.074   | -0.215 |
| PP  |     |         |        |         |         |        |         |         |         | 1       | -0.112 |
| SSB |     |         |        |         |         |        |         |         |         |         | 1      |

\*\*Correlation is significant at the 0.01 level (2-tailed); \*Correlation is significant at the 0.05 level (2-tailed); GYd: Grain yield (kg plot<sup>-1</sup>); DRY: Dry fodder yield; DF: Days to 50% flowering; DM: Days to maturity; PH: Plant height (cm); TN: No. of productive tiller; PL: Panicle length (cm); PD: Panicle diameter (cm); TSW: 1000 Seed weight (kg h<sup>-1</sup>); PP: Plant population (cm); SSB: Seed set under bagging

The days to maturity exhibited a substantial positive correlation with dry fodder output ( $r=0.543$ ) and days to 50% blooming ( $r=0.65$ ). Significant positive associations were noted between plant height and dry fodder weight ( $r=0.52$ ), days to 50% blooming ( $r=0.46$ ), and days to maturity ( $r=0.51$ ). Panicle length had a positive correlation with dry fodder weight ( $r=0.41$ ), days to 50% blooming ( $r=0.51$ ), and plant height ( $r=0.83$ ) (Table 1). Thousand seed weight exhibited a strong positive correlation with panicle diameter ( $r=0.56$ ). The inverse relationship between panicle length and the number of productive tillers plant<sup>-1</sup> ( $r=0.37$ ) might be ascribed to the crop's vulnerability to water stress during crucial growth phases, especially blooming and grain filling. Water stress at critical periods, such as panicle development, results in substantial yield losses. Pearl millet is particularly susceptible to dryness during the blooming and grain-filling stages, significantly affecting grain production and its components (Lahiri et al., 1996; Mahalakshmi et al., 1987; Mahalakshmi and Bidinger, 1985).

### 3.3. Expression analysis

The heat map and double dendrogram indicated diversity among the germplasm, with varying levels of expression based on morpho-physiological, yield, and yield-related traits. This analysis grouped thirty germplasm into two major clusters, I and II (Figure 1). Cluster I comprise six germplasm, which are further divided into two primary subclusters: aI and bI. Cluster II comprises twenty-four germplasm, which are further divided into two primary subclusters: aII and bII. Subcluster aII comprises twelve germplasm, including the widely recognised variety HHB299, which is a bio-fortified hybrid developed for

elevated iron and zinc content in the grain. Additionally, Dhanshakti and Kaverisuperboss serve as check varieties. This cluster encompasses potential germplasm for crop enhancement characterised by elevated iron and zinc content. Sub cluster bII comprises twelve germplasm. The second dendrogram of two-way clustering for quantitative traits revealed two primary clusters, labelled I and II. Clusters I included the nine traits, while the two quantitative traits, namely plant height and population at harvest, were categorised in cluster II, similar dendrogram analysis has been done earlier by Asati et al., 2022; Bhowmick et al., 2020; Yadav et al., 2023; Tiwari et al., 2023; Makwana et al., 2023; Rathore et al., 2022 (Figure 1).

### 3.4. Biochemical analysis

The nutritional composition of thirty diverse Pearl millet germplasm, comprising landraces and commercial varieties, was evaluated using standard protocols. The findings demonstrated significant variability in biochemical parameters (Sharma et al., 2021; Tomar et al., 2022; Sahu et al., 2020; Rana et al., 2023). Protein serves as a crucial macronutrient and a key component of tissues in both humans and animals. They serve as enzymes for metabolic pathways, facilitate maintenance and growth, function as hormones and signalling molecules, regulate physiological pH, support immunity, and store and transport molecules (Kumar et al., 2021). PM serves as a significant source of dietary proteins, containing essential amino acids (Dias-Martins et al., 2018). The protein content observed in this study varied from 9.2% in GHB 905 to 11.5% in HHB 299. The results were consistent with the findings of Buerkert et

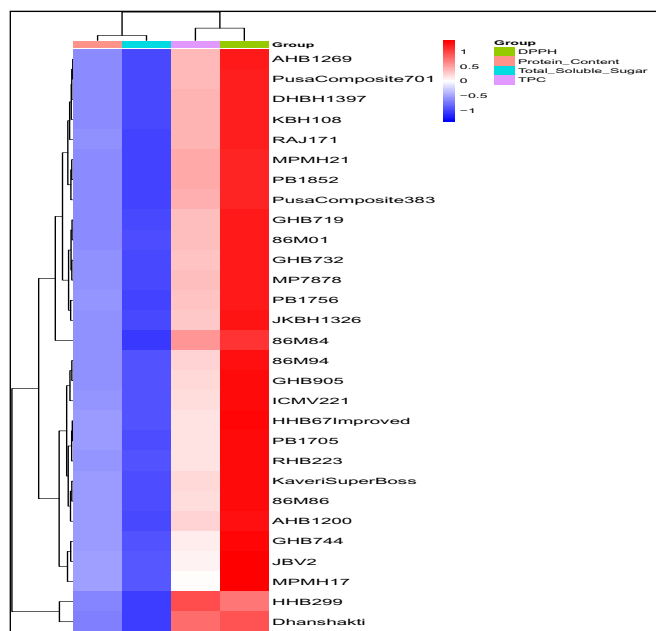


Figure 1: Heat map and double dendrogram showing diversity and variation in expression pattern of pearl millet genotypes based on morphological observations (GYd: Grain yield (kg plot<sup>-1</sup>); DRY: Dry fodder yield; DF: Days to 50% flowering; DM: Days to Maturity; PH: Plant Height (cm); TN: No. of Productive tiller; PL: Panicle Length (cm); PD: Panicle diameter (cm); TSW: 1000 Seed weight (kg h<sup>-1</sup>); PP: Plant Population (cm); SSB: Seed set under bagging)

al. (2001) and Abedi et al. (2011). The protein content is influenced by the genotype of cultivars and is also affected by environmental factors such as nitrogen application, soil moisture, and temperature, particularly during the grain filling period in pearl millet. The findings suggest that

increased protein levels in these grains may effectively address protein malnutrition in a cost-efficient way. Protein isolates derived from these grains can be utilised in the formulation of functional, protein-rich foods and food additives. The protein content varied between 9.2% and 11.5%, indicating notable differences in nutritional value across germplasm. Sugars are the primary factors influencing the glycaemic index of foods. The low sugar content in PM contributes to its hypoglycemic properties. The total soluble sugars (TSS) content in particulate matter (PM) ranged from 1.4 g 100 g<sup>-1</sup> in RAJ 1.71–2.6 g 100 g<sup>-1</sup> in MPMH 17. A comparable range of 2.16–2.78 g 100 g<sup>-1</sup> was noted by Subramanian et al. (1981), whereas Jambunathan and Subramanian (1988) reported a range of 1.4–2.6 g 100 g<sup>-1</sup> for Indian cultivars, and non-Indian cultivars were stated to have values of 1.4 and 2.78 g 100 g<sup>-1</sup>. Polyphenols represent the largest class of phytochemicals associated with numerous health benefits (Tian et al., 2020b). Numerous studies indicate that PM contains a high concentration of phenolic compounds, which exhibit significant antioxidant and metal chelating properties (Saleh et al., 2013). Total Phenolic Content (TFC) varied from 22.14 mg 100 g<sup>-1</sup> in MPMH 35 and MPMH 17–50.87 mg-100 g<sup>-1</sup> in HHB 299, suggesting antioxidant potential. El Hag et al. (2002) reported similar content, while Elyas et al. (2002) found values ranging from 0.29–0.31 g 100 g<sup>-1</sup> for non-Indian cultivars. The elevated phenol content in PM renders them effective for sustaining cellular redox potential and neutralising reactive oxygen species (ROS). DPPH Radical Scavenging Activity varied from 44.18% in MPMH 21–55.68% in DHBH 1397, indicating the diverse antioxidant capacities among the germplasm. A comparable range of DPPH% was

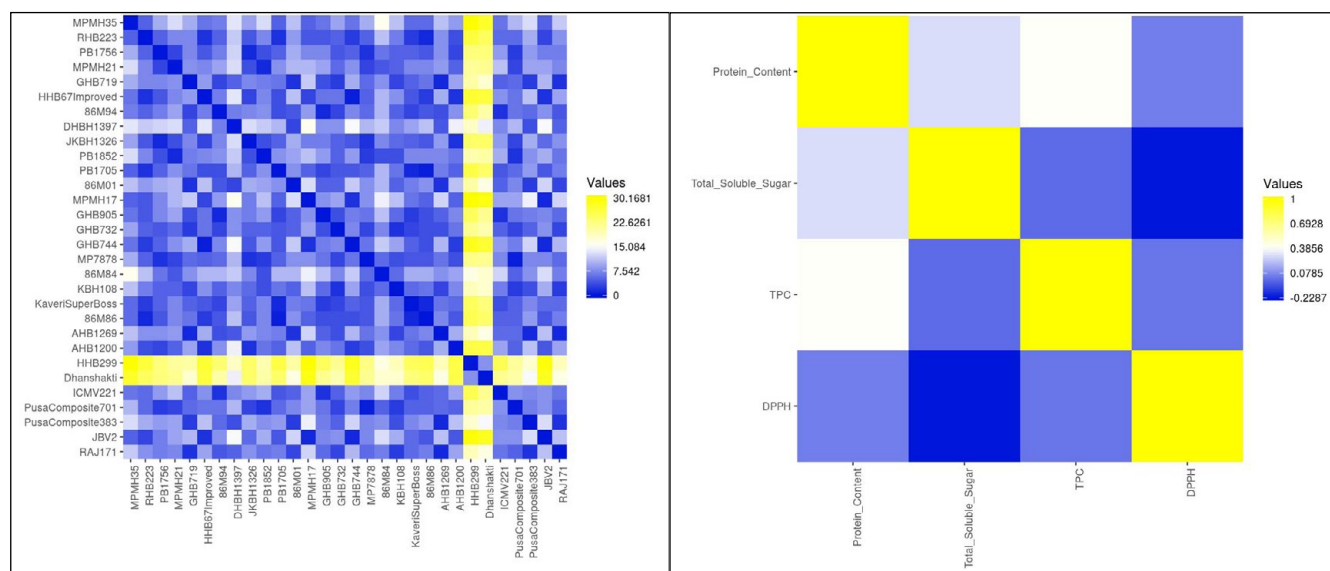


Figure 2: Correlation matrix and pairwise distance matrix representing correlation expression pattern and variations among the biochemical data



reported by Salar et al. (2017). The variations highlight the significance of biochemical profiling in the selection of germplasm that offer enhanced nutritional and health benefits (Table 3). This study highlighted the diversity among pearl millet germplasm for both morphological and nutritional traits. Germplasm exhibiting superior yield, and enhanced biochemical properties, such as higher protein and antioxidant content, were identified in HHB 299 and Dhanshakti. These findings can support the development of climate-resilient and nutritionally fortified pearl millet cultivars, which are crucial for improving food security and public health. The heatmap illustrates the biochemical diversity among 30 pearl millet varieties based on total soluble sugars (TSS), protein content, total phenolic content (TFC) and DPPH radical scavenging activity. Varieties such as HHB 299, DHBH 1397, and 86M84 clustered together, showing higher values across protein, TFC, and DPPH, indicating strong nutritional and antioxidant profiles (Figure 2). Dhanshakti also performed well, especially in protein and TFC. In contrast, genotypes like AHB 1269, JKBH 1326, and Pusa Composite 701 exhibited lower values across these parameters (Figure 3). Overall, the heatmap highlights considerable biochemical variation among the germplasm, identifying elite lines for future nutritional improvement programs (Figure 4).

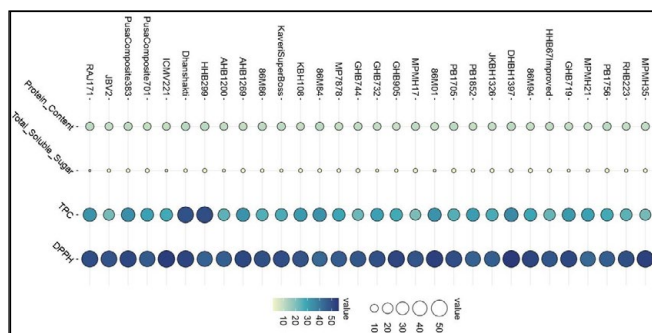


Figure 3: Balloon diagram representing values of proteins content, total soluble sugar, total phenolic contents and DPPH in pearl millet germplasms

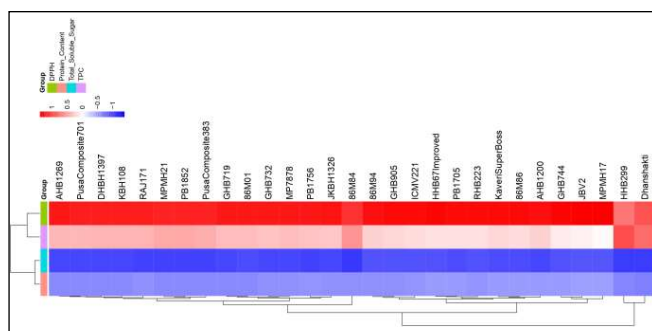


Figure 4: Heat map and double dendrogram representing expression pattern of proteins content, total soluble sugar, total phenolic contents and DPPH in pearl millet germplasms

Table 3: Biochemical estimation of pearl millet germplasm for total protein, total soluble sugar, total phenolic contents and DPPH

| Pearl millet variety name | Protein content (%) | Total soluble sugar (g 100 g <sup>-1</sup> ) | TPC (mg 100 g <sup>-1</sup> ) | DPPH (%) |
|---------------------------|---------------------|----------------------------------------------|-------------------------------|----------|
| MPMH 35                   | 10.50               | 2.10                                         | 22.14                         | 54.28    |
| RHB 223                   | 9.70                | 1.80                                         | 25.14                         | 50.14    |
| PB1756                    | 11.00               | 2.40                                         | 28.14                         | 46.14    |
| MPMH 21                   | 9.50                | 1.70                                         | 30.14                         | 44.14    |
| GHB 719                   | 9.90                | 1.60                                         | 32.14                         | 52.14    |
| HHB 67 Improved           | 10.30               | 2.20                                         | 24.14                         | 48.14    |
| 86M94                     | 9.80                | 2.50                                         | 29.14                         | 53.14    |
| DHBH 1397                 | 11.30               | 2.10                                         | 35.64                         | 55.68    |
| JKBH1326                  | 10.00               | 2.30                                         | 27.14                         | 46.14    |
| PB 1852                   | 9.30                | 1.90                                         | 31.14                         | 45.14    |
| PB 1705                   | 11.10               | 2.50                                         | 26.14                         | 51.14    |
| 86M01                     | 9.50                | 1.50                                         | 33.14                         | 54.23    |
| MPMH 17                   | 10.60               | 2.60                                         | 21.14                         | 49.14    |
| GHB 905                   | 9.20                | 1.80                                         | 28.14                         | 53.14    |
| GHB 732                   | 9.70                | 1.60                                         | 30.14                         | 50.14    |
| GHB 744                   | 10.40               | 2.30                                         | 23.14                         | 48.14    |
| MP7878                    | 9.90                | 2.00                                         | 29.14                         | 47.14    |
| 86M84                     | 11.20               | 2.10                                         | 34.14                         | 44.18    |
| KBH 108                   | 9.60                | 2.20                                         | 32.14                         | 49.14    |
| Kaveri super boss         | 10.90               | 1.70                                         | 27.14                         | 51.14    |
| 86 M 86                   | 11.00               | 1.90                                         | 26.14                         | 50.14    |
| AHB 1269                  | 10.20               | 2.40                                         | 33.14                         | 52.14    |
| AHB 1200                  | 10.00               | 1.60                                         | 25.14                         | 46.14    |
| HHB 299                   | 11.50               | 2.50                                         | 50.87                         | 45.14    |
| Dhan shakti               | 11.40               | 1.90                                         | 49.58                         | 53.14    |
| ICMV 221                  | 9.80                | 1.50                                         | 28.14                         | 54.89    |
| Pusa Composite 701        | 9.50                | 2.30                                         | 30.14                         | 47.14    |
| Pusa Composite 383        | 10.80               | 2.00                                         | 34.78                         | 52.14    |
| JBV 2                     | 9.90                | 1.80                                         | 22.14                         | 49.14    |
| RAJ 171                   | 11.10               | 1.40                                         | 33.14                         | 51.14    |
| Mean                      | 10.29               | 2.01                                         | 30.08                         | 49.79    |
| CD ( $p=0.05$ )           | 0.46                | 0.09                                         | 1.22                          | 2.33     |
| SE(m)                     | 0.16                | 0.03                                         | 0.43                          | 0.82     |
| SE(d)                     | 0.23                | 0.05                                         | 0.61                          | 1.16     |
| C.V.                      | 2.75                | 2.75                                         | 2.47                          | 2.86     |

# 4. CONCLUSION

This study demonstrated significant genetic diversity among 30 pearl millet germplasm for agronomic and biochemical traits. Notable variations were observed in grain yield, dry fodder yield, flowering time, and plant height, along with biochemical properties like protein, total soluble sugars, phenolic content, and antioxidant activity. Germplasm like HHB 299 and Dhanshakti showed exceptional yield and nutritional traits, highlighting their breeding potential. This diversity emphasizes the value of diverse pearl millet germplasm in breeding programs to develop high-yielding, nutritionally rich, and resilient cultivars, ensuring food security in challenging climates.

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