Morpho-biochemical characterization and molecular ~~marker~~ marker-based diversity study in pearl millet [*Pennisetum glaucum* (L.) R. Br.]

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Abstract

Pearl millet is a key food for millions living the semi-arid and arid regions. The poor's diet contains more pearl millet than other grains. The genetic diversity existing in the pearl millet germplasm can be used to improve the micronutrient content and grain yield. Effective and organized exploitation of diversity at morphological and DNA levels is the ~~strategic~~ strategy ~~to~~ for any crop improvement program. In this study, the genetic diversity of 48 pearl millet genotypes ~~were~~ was evaluated for eight morphological traits and eleven biochemical characters. Genotypes were characterized using twelve SSR and six SRAP markers. ~~Significant~~ The significant mean difference ~~among~~ between morphological and biochemical was detected in ANOVA. The productive tillers per plant varied from 2.65 to 7.60 with a mean of 4.80. ~~Grain~~ The grain yield of genotypes varied more than 3x from 15.85 g (ICMR 07222) to 56.75 g (Nandi 75) with an average of 29.54 g per plant. During the experiment higher protein, iron, and zinc ~~was~~ were recorded in ICMR 12555 (20.6 %), ICMR 08666 (77.38 ppm), and IC 139900 (55.48 ppm), respectively. Substantial variability was observed for grain calcium as it ranged from 100.00 ppm (ICMR 10222) to 256.00 ppm (ICMR 12888). The top eight nutrient-dense genotypes flowered in 34–74 days and had 5.71–9.39 g 1000 grain weight. Genotype ICMR 08666 was dense for Fe, Zn, K, and P. The inter-genotype similarity coefficient at the genetic level, generated using DNA markers, ranged from 0.616 to 0.877 with a mean of 0.743.

Açıklamalı [Ref1]: Alternative title: Morpho-biochemical characterization and molecular marker-based diversity of a panel of pearl millet [*Pennisetum glaucum* (L.) R. Br.] accessions

Açıklamalı [Ref2]: morphological and biochemical….what?

Açıklamalı [Ref3]: content?

Açıklamalı [Ref4]: May not be the right term.

~~Combination~~ A combination of phenotypic and genotypic diversity carried out in this research will help to differentiate the genotypes under study in a better way.

***Key-words*:** Diversity, Germplasm, Grain minerals, Pearl millet, Variability, Yield

1. Introduction

Pearl millet [*Pennisetum glaucum*(L.) R. Br.] is a small-seeded C4 plant of the Poaceae family. The 1.7 GB genome of this crop is accommodated by 2n=2x=14 chromosomes. Compared to other cereals like wheat and rice, it can withstand effectively ~~under~~ drought, nutrient-depleted soil, and ~~high~~ high-temperature conditions of the hot deserts of India~~n~~ and Africa~~n~~. This hardy nature makes pearl millet ~~a~~ resilient to harsher climatic consequently it is cultivated in marginal environments of arid and semi-arid tropical regions (Ramya et al., 2018). Pearl millet is a key food for millions living the semi-arid and arid regions. The grain is mainly consumed as ~~a~~ human food while biological yield is used as livestock feed. ~~The p~~Pearl millet is a primary food for mankind living in dryland agriculture regions.

Pearl millet accounts for circa 50% of the total global millet production. It is grown on >28 million ha, mainly in Africa and India. India is the world's largest producer of millets, harvesting 11 million tonnes annually, or around 36% of global production. In 2020, India harvested 8.61 million tonnes of pearl millet grains from a 6.93 million ha area with 1,243 kg/ha of productivity (Directorate of Millets Development, 2020) (AICPMIP, 2020).

In any breeding strategy, variation continues to be the key to success. Pearl millet shows abundant phenotypic variability for most of the quantitative traits like flowering time, ear head length, grain characteristics, tolerance to various (a)biotic stresses as well as nutritional quality (Bhattacharjee et al., 2007). Effective and logical utilization of this diversity is ~~the~~ vital to any breeding program (Allard et al., 1960). Exploiting this genetic diversity in the pearl millet population will allow the improvement of micronutrient~~s~~ density in grain and grain yield.

Micronutrient insufficiency has emerged as a global problem, particularly for those living in underdeveloped nations and consuming carbohydrate ~~rich cereal~~ rich cereal-based diets. This deficiency can be managed with pearl millet, a nutritious cereal. Among all coarse cereals, pearl millet grains are dense in minerals like Fe and Zn concentration and essential amino acids. In

Açıklamalı [Ref5]: This sentence does not highlight the significance of the research. The results should be interpreted better with the advantage of the most important traits.

Açıklamalı [Ref6]: It seems that preposition use may be incorrect here.

Açıklamalı [Ref7]: "high-temperature conditions of the hot deserts" ?

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Açıklamalı [Ref9]: "semi-arid tropical" which tropic areas are arid or semi-arid?

May be sub-tropic?

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comparison to wheat (11.8 g/100 g), the protein in this coarse cereal ranges between 9 - 21%, which is higher than sorghum (10.4%), rice (6.8%), and maize (4.7%) (Kaur et al., 2014). ~~The~~ ~~g~~Gluten-free grains have a low glycemic index. The provitamin-A enriched grains are also a richer source of fat (5-7 g/100 g) but are scarce in vitamins B and C (Gopalan et al., 2003). Pearl millet grain is encased in a tough fibrous seed that contains a variable amount of inhibitory factors like phytic acid and polyphenols (Arora~~-~~ et al., 2003). But these factors can be reduced through various approaches like soaking, fermentation, blanching, and roasting (Kaushik and Grewal, 2017) up to a certain ~~extend~~ extent only. Moreover, the presence of anti-nutrients hinders the biofortification in millet.

To improve the nutritional quality and diminish the anti-nutritional factors of pearl millet through any breeding approach, ~~the~~ knowledge about the variability for mineral content, anti-nutritional factors, and their relation with yield is a prerequisite. Accumulation of both micro- and anti-nutrients in seeds is a complex mechanism containing numerous genes and is affected by the environment (Anuradha et al., 2017). Due to the confounding effect of the environment, similar phenotypes may have different genomic constituents. Therefore, it is hard to equate morpho-biochemical and genetic variability. In contrast, molecular markers reflect the authentic genetic variability and relationships among accessions than phenotypic markers (Glaszmannet al.2010). In pearl millet, microsatellite, SNP and RFLP markers have been applied to create linkage maps followed by quantitative trait loci mapping and germplasm characterization (Kumar et al., 2016). The density and genome coverage can be improved by the combination of various markers. In pearl millet, no report is available on the deployment of sequence-related amplified polymorphism (SRAP) marker. Therefore, in the current experiment, both SRAP and microsatellite markers have been used to expose the genetic diversity. SRAP markers are dominant markers that target genomic coding sequences and have been employed for genetic diversity assessment (Li and Quiros, 2001). With the purpose to contribute to upsurging the nutritional quality and the food safety of pearl millet as well as widen understanding in this area, the current study was designed to analyze the natural variability for grain mineral and anti-nutrient~~s~~ content in grain along with molecular diversity in pearl millet germplasm.

Açıklamalı [Ref11]: Is the gluten free status of pearl millet mentioned before in this text?

Açıklamalı [Ref12]: This term is not given previously in this text and is not a common word to know. Please explain any term that is not in common knowledge.

Açıklamalı [Ref13]: What are they?

Açıklamalı [Ref14]: ? Would not this be opposite?

Similar genotypes would have different phenotypes due to environmental variability.

Açıklamalı [Ref15]: I am not sure abut this...

https://acsess.onlinelibrary.wiley.com/doi/full/10.2135/cropsci2009.10.0560

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2. Materials and methods

With two replications, the field trial was done in a randomized complete block design (RBD) ~~with two replications~~. The inter- and intra-row distance was 60 and 15 cm, respectively. The recommendations for crop management practices were followed for uniform plant growth and ~~the~~ a healthy crop stand. The seeds were sown on February 2021. A total of 48 pearl millet genotypes were used for the study (Supp Table 1).

2.1. Morphological characters

At the panicle emergence stage, panicles were covered with glassine bags to prevent cross-pollination by outside pollen and to collect self-seeds. For phenotyping of grain traits, physiologically mature panicles were collected, dried under sunlight, and then manually threshed in bulk.

The experimental material was evaluated for eight morphological traits viz., days to 50% flowering, plant height, panicle diameter (PD, mm), panicle length (PL, cm), number of productive tillers, grain yield, days to maturity, and 1000 grain weight. Except for days to 50% flowering and days to maturity, which were recorded on a plot basis during the study, data on the above traits were collected from randomly tagged five competitive plants in each genotype in both replications. PL was measured with Vernier calipers on panicles for which PL was recorded.

2.2. Biochemical characters

Before biochemical analysis, grains were cleaned followed by hot air oven drying (80°C for 24 h). Dried grains (10 g) were powdered manually. For mineral analysis, 0.5 g flour was processed in 11 mL of nitric acid (69%) and 1 mL of H_2O_2. The digestions were carried out in HVT50 vessels using rotor 12HVT50 in Multiwave GO/ Multiwave GO plus. For microwave digestion, the initial temperature was kept at 180°C with a ramp of 20 minutes and a hold period of 12 minutes. While the second round of digestion was performed with a ramp time of 10mins ~~on~~ at 70°C temperature and a holding time of 5 minutes. Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES (model 7000DV of make: Perkin Elmer, made in USA with wintab32 software ver. 5.1) was used to determine the mineral content (Fe, Zn, Ca, Cu, and Mn) in grain after diluting the digested mixture to a volume of 50 ml using distilled water. The flow

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Açıklamalı [Ref21]: Yield components?

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rate in the peristaitic pump was 1.5ml / min. From the acid extract, potassium content was quantified using a flame photometer (Jackson, 1973), while the vanadate-molybdate method of Jackson (1973) was used to estimate the phosphorus. Total phenols ~~was~~ were estimated using the Folin-Ciocalteau reagent as Malik and Singh (1980) and reading was measured at 730 nm using a spectrophotometer. Soxhlet extraction was performed to estimate the crude oil content, and semi-micro-Kjeldahl was employed to determine the crude protein content.

2.3. Molecular marker study

Genomic DNA was extracted from tender leaves as per Mace et al (2003). Genotyping was done using SSR and SRAP markers. For microsatellite marker profiling, markers from the PMES series were amplified in SensoQuest Thermocycler (Germany). The SSR-PCR reaction conditions were as follows: 94°C (initial denaturation) for 5 min., followed by 35 cycles of 94°C for 45 sec, X°C (primer specific) for 45 sec, 72°C for 45sec, and 72°C for 7 min (final extension)~~.~~. The SRAP amplification ~~were~~ was as follows~~.~~: 94 °C (initial denaturation) for 5 min., followed by 5 cycles of 94 °C for 30 s, 35°C for 45 s, and 72°C for 90 s, followed by 35 cycles of 94°C for 30 s, X°C (primer specific) for 45 s, 72 °C for 60 s and 72 °C for 10 min (final extension)~~.~~. ~~A~~ An agarose gel (3%) was used to resolve PCR products.

2.4. Statistical analysis

The mean value of traits was figured out, and analysis of variance (ANOVA) was performed in accordance with Panse and Sukhatme (1978). ~~Phenotypic~~ A phenotypic ~~trait~~ trait-based dissimilarity matrix was constructed using Manhattan coefficients with NTSYSpc 2.0 (Rohlf 1998). The amplified products of SSR and SRAP markers were scored in 1 (presence) and 0 (absence) fashion. Polymorphism information content (PIC), Multiplex ratio (MR), effective multiplex ratio (EMR) marker index (MI), and resolving power (Rp) value ~~were~~ were estimated following Sharma et al. (2016). In NTSYSpc 2.0, The SIMQUAL program used ~~the~~ Jaccard's similarity (J) coefficient to compute the genetic similarity between genotypes, SAHN clustering method was used to construct the UPGMA dendrogram.

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3. Results and discussion

3.1. ANOVA

Genetic variability, a quantitative measure of genetic dissimilarities, is the total ~~of~~ genetic diversity within a population. The genetic differences among genotypes are the result of natural selection over a period of time. Greater variability present in the base breeding germplasm ensures good opportunities to produce desired genetic architecture of a plant. The ANOVA resulted that genotypic variations were very significant for all the traits, showing ~~an~~ ample genetic diversity among the genotypes under study. (Table 1). This also suggested that there is sufficient scope to select superior breeding material which can be exploited in pearl millet breeding programs.

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Açıklamalı [Ref29]: introduction

Açıklamalı [Ref30]: what does very mean? Not a specific measure.

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3.2. Character variance analysis

3.2.1. Morphological parameters

Early flowering is a desirable trait for pearl millet as it is a crop of (semi)-arid regions. Earliness becomes an important trait in areas where scanty and erratic rains aggravate the moisture stress condition during the growth stage of the crop and leads to ~~a~~ post-flowering moisture stress (Yadav et al., 2011). In the current study, though, the population mean for days to 50% flowering was 54.69 days but the days to 50% flowering ranged from 34 (IC 370523) to 77 days (ICMR 07999). Earlier literature also recorded similar values for days to 50% flowering for example 49.06 days by Govindaraj et al. (2011), 53.10 days by Sonali et al. (2019), and 55.61 days by Pallavi et al. (2020). PH is an important trait that governs tradeoffs between competition and resource distribution, which is decisive for productivity (He et al., 2021). Semi-dwarf genotypes are better than their tall counterparts because of their reduced lodging vulnerability and better response to nitrogen Azhaguvel et al. (2003). In the present experiment, PH ranged from 110.10 cm (ICMR 06555) to 205.35 cm (IC 332715) with an average of 149.42 cm. The results indicated that most of the studied genotypes are semi-dwarf in nature and with better management dwarfism supports the grain yield. Panicle size (length and diameter) are two important traits ~~which~~ that have direct positive correlations with grain yield in pearl millet (Vengadessan et al., 2013). Hence, the improvement of sink-size relation traits is a key objective for pearl millet improvement programs.

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PL in the present study ranged from 15.55 cm (ICMR 11888) to 38.05 cm (IC-332716) with an average of 24.03 while the diameter ranged from 1.03 cm (ICMR 10999) to 2.15 cm (ICMR 09333) with an average of 1.53 cm. Abubakar et al. (2019) observed a similar range and mean in pearl millet (2.26 cm) for panicle diameter. Similarly, results for PL are comparable with previous reports (Sharma et al., 2018, Rani et al., 2019). The number of productive tillers per plant varied from 2.65 (ICMR 08222, ICMR 11999) to 7.60 (IC 370523) with an average of 4.80 (Table 2). According to Sile et al. (2004), non-tillering millet genotypes produced bold seeds than the genotype that produced tillers. Similarly, Maman et al. (2004) also reported that, a reduction in productive tillers from 10 to 3 or 5 improved seed yields by 15-30%. Yadav et al (2021) reported that ~~private~~ private-sector hybrids are generally having less ~~number of~~ effective tillers/plants. But still, farmers in drought-prone areas prefer high tillering hybrids because tillering is a strategy of adaptation to intermittent drought spells (Yadav et al., 2016).

In cereal breeding, yield is one of the supreme traits which is influenced by several associated traits. This makes grain yield a complicated trait. ~~Grain~~ The grain yield of genotypes varied more than 3x from 15.85 g (ICMR 07222) to 56.75 g (Nandi 75) with an average of 29.54 g per plant. Large variability was also observed for 1000 grain weight which is determined by the form, size, and density of the grain and these are directly related to total grain yield. TGW ranged from 4.93 g (ICMR 06888) to 10.45 g (ICMR 06555) with an average of 7.14 g. A diversity assessment of 21,594 pearl millet genotypes from 50 nations revealed huge variability for the 1000-seed weight (1.5 to 21.3 g) (Upadhyaya et al., 2007). Three-fold variability for TGW (6-16 g) was earlier recorded by Pujar et al (2018).

3.2.2. Biochemical parameters

~~Comparing~~ Compared to other main cereal crops, pearl millet yields nutritious grains that are a great source of protein, calcium, phosphorus, iron, and zinc (Devos et al., 2006). Currently, the commercially grown varieties/hybrids of pearl millet produce grains with an average Fe and Zn content of 42 and 32 ppm, respectively (Rai et al. 2016). However, a much wider variability for these micro-nutrients has been reported in germplasm collections (Rai et al. 2014). Iron is an essential element for blood production and for the growth and development of the body. Zinc is essential for the development of a strong immune system. The values of iron content in the current study ranged from 31.58 (ICMR 07777) to 77.38 (ICMR 08666) with an average of 49.69. Zinc content ranged from 29.34 (ICMR 08111) to 55.48 (IC 139903) with an average of

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Açıklamalı [Ref36]: Others are nutrititious as well, pearl millet may have higher content in some nutrients or minerals

39.36 (Table 2). A similar mean value ~~were~~ was observed by Velu et al. (2007; Fe: 45.50), Anuradha et al. (2017; Fe: 57.65, Zn: 46.61), Anuradha et al. (2018; Fe: 55.73, Zn: 42.75), Sonali et al. (2019; Fe: 53.57, Zn: 40.39) and Yadav et al. (2020; Fe: 50.60, Zn: 38.60).

In the human body, fats and carbohydrates metabolism, absorption of Ca, and the control of blood sugar are all impacted by manganese. It is also essential for standard brain/nerve functioning and bone mineral density. The values of manganese content ranged from 7.20 ppm (ICMR 07222) to 17.63 ppm (ICMR 08444) with an average of 14.04 ppm. The outcome is in congruence with Anuradha et al. (2017), Kumar et al. (2020), and Govindaraj et al. (2020a). Similarly, a low value of Mn (8 ppm) was recorded by Oshodi et al. (1999).

Calcium is very important for the contraction of muscle, the development of strong bones and teeth, blood clotting, the transmission of nerve impulses, and in the ~~regulating~~ regulation of heart beat in cells. This is claimed that a high intake of cereal grains increases the chances of calcium deficiency. But this is not true with pearl millet as substantial variability was observed for grain calcium as it ranged from 100.00 ppm (ICMR 10222) to 256.00 ppm (ICMR 12888) with an average of 199.31 ppm. Higher variability for Ca (85-249 ppm) was also recorded by Govindaraj et al. (2020b) in pearl millet core collection. In the current study, 50% of the genotypes had high calcium (>200 ppm).

Copper is essential for the synthesis of elastin and collagen. It is a key cofactor of many metalloenzymes playing role in metabolism Fe and cellular respiration. In the current study, the grain Cu ranged from 4.92 ppm (IC 332703) to 22.59 ppm(GHB 558) with an average of 9.86 ppm. The range of grain Cu in various studies ~~are~~ is different like 4.14-15.35 in Anuradha et al. (2018), 4-7 ppm in Govindaraj et al. (2020), 3.19-4.76 ppm in Warrier et al (2020).

The transport of water, nutrients, and carbohydrates within plant cells is linked with potassium. It is a crucial mineral for the activation of several enzymes that control the synthesis of protein, starch, and adenosine triphosphate (ATP) in plants. The potassium ranged ~~from1800ppm~~ from 1800 ppm (ICMR 07222) to 6000 ppm (ICMR 10999) with an average of 4700 ppm. Large variability for potassium was also recorded in 122 commercial pearl millet cultivars (3675–5375ppm; Govindaraj et al. 2020a) and core collection(3667-5133ppm; Govindaraj et al. 2020b).

Biçimlendirilmiş: Gövde Metni, Sola, Satır aralığı: tek

Açıklamalı [Ref37]: First sentences of each paragraph define the importance of essential minerals in the body. They may be mentioned in the introduction to express the importance of the study. But not in here. These kinds of sentence extend the text beyond its scope. Please revise these several paragraphs.

The body needs phosphorus to produce protein for the development, upkeep,~~,~~ and repair~~ing~~ of cells and tissues. Additionally, it participates in the production of ATP. The values of phosphorus content ranged from 2200 (IC 139900) to 3600 ppm (ICMR 08666) with an average of 3112 ppm. ICMR 06555 was statistically at par with IC 139900.

Pearl millet is also a promising source of protein. Studies indicated that protein in pearl millet is circa 11.8 %, which is better than rice (8.6 %), and maize (9.2 %) and comparable with sorghum (10.7 %). Moreover, pearl millet grain is enriched with glutamate which is a precursor of γ-aminobutyric acid (GABA) (Tomar et al., 2021). In the current study, the protein content ~~in~~ ranged from 8.26% (IC 332716) to 20.06% (ICMR 12555) with an average of 13.73%. ICMR 07444 (9.89%) was statistically at par with IC 332716. The study of Pujar et al. (2020) reported grain protein content variation between 6 - 18%, with a mean of 11% while it varied between 8.5-15.1% in the report of Abdalla et al. (2007). The augmentation of pearl millet in daily food can reduce the risk of protein malnutrition in an economical way. Moreover, protein extracted from pearl millet can be exploited to design protein-enriched functional foods.

The lipid content ranged from 2.72% (ICMR 06999) to 6.95% (ICMR 08444) with an average of 4.68%. A comparable range and mean were observed by Arulselvi et al. (2007; 5.12%), Abdalla et al. (2007; 2.70-7.10%), and Tomar et al. (2021; 5.24-9.99). The lipid content in pearl millet was 1.5 times higher sorghum and corn. Though, the high lipids have been documented as possible causes for the rancidity of millet flour. However, the shelf life of flour can be increased by hydrothermal treatment, irradiation, cooling storage, or a combination of more than one technology. (Goyal and Chugh, 2017)

The ~~metal~~ metal-chelating ability of phytic acid makes it ~~is~~ an antinutritional hytochemical as it declines the bio-availability of ions like Mn, Ca, Mg, Fe, and Zn (Marathe et al., 2018). In the current study, the phytate ranged from 201.5 mg/100g (ICMR 08111) to 542.50 mg/100g (GHB 558) with an average of 282.39. A similar range was detected by Abdalla et al. (2007; 354-795). The study ~~of~~ by Gabaza et al. (2018) reported that phytate in pearl millet grains ranges between 580 mg/100g to 1380 mg/100g which is similar to sorghum and maize. The range of phytate in the current study is supported ~~with~~ by the result of Pushparaj and Urooj (2014) in Indian cultivars where it was between 0.26 - 0.99 g/100 g. The result suggested that phytic acid content

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in pearl millet grain is significantly lower than in rice, oat, soybean, and wheat. Hence, regular consumption will possibly not hamper the bioavailability of minerals.

Polyphenols have many health benefits as having antioxidant activity. Moreover, phytic acid is considered to be beneficial in dropping cholesterol and ~~reduces~~ reducing cancer risk. The values of total phenolic acid ranged ~~with~~ from 75.16mg/100g (ICMR 12555) to 44.41mg/100g (Nandi 75) an average of 60.26mg/100g. Higher phenol content in grain makes pearl millet a good food to maintain the redox potential of cells and to quench the ROS species. Phenolic may be particularly important in the treatment of postprandial hyperglycemia since it has been documented that it reduces intestinal ~~-~~-glucosidase and pancreatic ~~-~~-amylase (Shobana et al. 2009).

3.3. Nutrient-dense genotypes

Genotypes dense in multiple nutrients can directly be released as a variety after ~~evaluation~~ evaluating ~~its~~ their yield performance over the locations for multiple years. Such genotypes can be exploited in a hybridization program. In the current study, the top eight nutrient-dense genotypes flowered in 34–74 days and had 5.71–9.39 g TGW (Table 3). Top genotypes had Fe content of 61.07-77.38 (ICMR 08666) ppm, Zn content of 45.11-55.48 (IC 139900) ppm, Mn content of 16.2-17.63 (ICMR 08444) ppm, Ca content of 230.5-256 (ICMR 12888) ppm, Cu content of14.32-22.59 (GHB 558) ppm, Kcontent of52.3-60.15(ICMR 10999) ppm, and P content of 33.28-36.72 (ICMR 08666). IC 139900 was superior for both Fe (71.22 ppm) and Zn (55.48 ppm). Genotype ICMR 08666 was dense for Fe, Zn, K, and P. Out of eight high-Fe genotypes, only two genotypes had > 75 ppm. Thus current experiment also identified the best genotypes that had a higher content of multiple nutrients. Earlier, Govindraj et al (2020) also identified genotypes having a high content of multiple nutrients.

3.4. Phenotypic diversity analysis

Phenotypic diversity is important for pearl millet breeding. The interactions between the genome and all of its growing micro- and mega-environments lead to the phenotype of the plant (Fasoula et al., 2020). The mean value of each trait was used to generate the Manhattan dissimilarity coefficient and dendrogram (Sokal and Michener, 1958) using NTSYS-pc 2.02 program~~me~~ (Rohlf 1998). The genotypes were divided into nine major clusters based on the Manhattan dissimilarity coefficient. The average dissimilarity value among genotypes was calculated to be 0.16, demonstrating modest phenotypic variability (Table 4). The dissimilarity between

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Açıklamalı [Ref40]: What is the purpose of this sentence?

genotypes ranged from 0.08 (IC 139899 and ICMR 07888) to 0.27 (Nandi 75 and ICMR 07222) showing the maximum and minimum similarity for the respective pair of genotypes.

Cluster I comprise seven genotypes, characterized by high values of DFF, days to maturity, lipid, potassium, and Low values of NPT. Cluster II consists of ~~twenty~~ twenty-five genotypes. Cluster III contains four genotypes, namely ICMR 10222, Nandi 75, ICMR 12111, and ICMR 10888. This cluster is characterized by more GY, days to maturity, and 1000 grain weight. Cluster IV has four genotypes, namely ICMR 08999, ICMR 11888, ICMR 139900, and ICMR 07777. This cluster is characterized by more lipid content, manganese content, and days to maturity. Cluster V has three genotypes (IC 332716, GHB 558, ICMR 07444) which ~~is~~ are characterized by the low value of head diameter, and lipid content. Cluster VI consists of two genotypes (ICMR 08111, and ICMR 12333). This cluster is characterized by more ~~number of~~ productive tillers per plant with low content of zinc. Cluster VII has only one genotype (ICMR 12555) which has high values for characters like days to maturity, protein content, manganese content, and ~~1000~~ 1000-grain weight (Table 4).

3.5. *Molecular study*

~~During~~ In recent days molecular markers have been commonly ~~using~~ used for assessing genetic diversity. The combined use of two or more markers (both dominant and codominant) for genetic diversity study has been better than the individual marker system. Molecular markers help overcome the limitation of morphological, biochemical, and protein-based markers which are affected by genotype x environmental interaction. The calculation of genetic diversity helps in the characterization of germplasm apart from crop improvement.

Forty-eight genotypes of pearl millet were ~~analysed~~ analyzed using SSRs and SRAP markers (Table 5). Out of 50 SSR, 32 (60%) primers showed amplification. 12 (37.5%), of 30 SSR primers certified polymorphic. The polymorphic SSRs generated 65 alleles with band sizes ranging from 85bp (PMES 190) to 292bp (PMES 171). Earlier, amplicon size from 101 to 285 bp was reported by Zala et al. (2017). The number of polymorphic bands ranged from 2 to 13, with a mean of 6.71. Zala et al. (2017) reported 2.20 alleles. The percentage polymorphism is 100%. The PIC for the marker was computed for the estimation of marker allelic variation based on allele frequencies in genotypes. PIC designates the informativeness of a primer. The mean

Açıklamalı [Ref41]: Would not this be minimum and maximum? Since 0.27 dissimilarity is the maximum dissimilarity observed.

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Açıklamalı [Ref43]: unnecessary

PIC was 0.28 on average and varied from 0.132 (PMES 171) to 0.499 (PMES 173). This range was comparable with Zala et al. (2017; 0.188 to 0.375).

The Rp was estimated considering the proportion of genotypes containing the amplicon. The primer that might best differentiate the cultivar can easily be identified by the value of Rp and PIC. In the current study, Rp varied from 1.625 (PMES 162) to 2.375 (PMES 168), with an average of 1.98. Mean Rp was between 0.154 (PMES 171) to 1.000 (PMES 173). The PI ranged from 0.998 (PMES 173) to 1.963 (PMES 168), though the mean PI value was 1.420. MI is considered to be an inclusive measure of the efficiency to detect polymorphism. The SSR MI was 19.092.

In the case of SRAP markers, of 25 SRAP, 6 (24%) were polymorphic. The polymorphic SRAPs amplified 119 amplicons. The product size for SRAPs ranged from 94 (Em2+Me2) to 1357 bp (Em6+Me3). The polymorphic bands ranged from 10 (Em6+Me3) to 34 (Em2+Me2), with a mean of 21.67. Liu et al. (2008) observed a polymorphic band detected with each ranging from 6 to 17, with an average of 11.76. Bhatt et al. (2017) had a band size from 120 to 500 bp. PIC oscillated from 0.224 (Em5+Me4) - 0.324 (Em6+Me2), with an average of 0.26. Bhatt et al. (2017) reported a PIC value (0.34).

The mean PI of SRAP was 5.69, through the maximum PI was for Em6+Me2 (8.108) and the lowest value ~~from~~ for Em6+Me3(2.335). Rp ranged from 4.625 (Em5+Me4) to 13.250 (Em6+Me2), with an average value of 8.27. Mean Rp was between 0.272 (Em5+Me4) - 0.542 (Em6+Me3) with an average of 0.40. Liu et al. (2008) stated higher RP values ranged from 2.229 to 8.457 with an average of 4.927. Fraction of polymorphism, MR, EMR, and MI for SRAPs are 1.00, 10.00, 10.00, and 15.65, respectively (Table 6).

3.6. Inter-Genotype Genetic Relationship

Forty-eight pearl millet genotypes were divided into seven major groups by the dendrogram created using pooled data from SSR and SRAP markers based on Jaccard's similarity matrix. Cluster I, II, III, IV, V, VI, and VII had ~~with~~ 1, 1, 11, 8, 3, 22, and 2 genotypes, respectively (Figure 1). In the study of Nehra et al. (2017), with SSR markers, 49 accessions were clustered into eight core clusters. Kumar et al. (2020) alienated 18 lines into three clusters in pearl millet using 74 SSRs. In the current study, the inter-accession genetic coefficient of similarity ranged

Açıklamalı [Ref44]: confusing. Should be re-organized.

Biçimlendirilmiş: Gövde Metni, Sola, Satır aralığı: tek

Açıklamalı [Ref45]: Why is it very low? Does it anything to do with the SRAP selection criteria?

Açıklamalı [Ref46]: No details are given for the discussed articles. How will reader, make a comparison and justification between this and relevant studies. Please provide necessary details of the articles when discussing. This comment applies to entire discussion section.

Açıklamalı [Ref47]: markers

from 0.616 to 0.877 while the average similarity was 0.743. ICMR 098888 and GHB 905 has a genetic distance (0.384) indicative that both genotypes are having moderate genomic difference level and can be crossed to create a bi-parental mapping population. The minimum genetic distance (0.123) was between IC 139899 and IC 332727, demonstrating that these accessions have more similarity in microsatellite locus. Moreover, on the based of on diversity results, breeders can select diverse genotypes for combining ability and heterosis analysis for traits studied in the current study. of

4. Conclusion

The genetic diversity for morphological and grain biochemical traits, an outcome of natural selection with the cross cross-pollination nature of pearl millet, was revealed by analysis of variance. Variability for grain micronutrient content was found greater with a wide range in the population. Genotypes ICMR 08666 and IC 139903 were superior for iron and zinc content, respectively. Genotype ICMR 08666 was also found promising for Zn, K, and P content and can further be utilized for genetic biofortification. In the present study, phenotypic diversity analysis grouped all genotypes into nine different clusters. Among all clusters, three clusters were shared only a single genotype with better phenotypic value for most of the grain biochemical parameters. But phenotype is a total outcome of the genotype and its interaction with the environment. Genetic markers are found effective in this study, they help to identify ICMR 098888 and GHB 905 as diverse genotypes for making a bi-parental mapping population.

5. Conflict of Interest

All authors declared that the research was conducted in the absence of any commercial or financial relationship that could be construed as a potential conflict of interest.

6. Authors' Contribution

SK, MPP, AAS & VK: Conceptualization & Designing the experiment, **DFG, RP & DK**: performed the experiments, **RP & SK**: analyzing the data, **VK:** Contribution of experimental material, **DFG, SK & RP**: Prepared the figures and tables, **SK & AAS**: Critically revised the manuscript for important content,

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References

Abdullah, M., A. Rehman, N. Ahmad, I. Rasul (2007). Planting time effect on grain and quality chacteristics of wheat. *Pak. J. Agri. Sci.*, 44(2), 200-202.

Abubakar, A., Falusi, O. A., Adebola, M. O., Olayemi, I. K., & Daudu, O. A. Y. (2019). Genetic diversity studies for morphological traits in pearl millet (*Pennisetum glaucum* L.) landraces of Northern Nigeria.

Allard, R. W. (1960). *Principles of Plant Breeding*. New York: John Willey and Sons. Inc

Anuradha, N., Satyavathi, C. T., Bharadwaj, C., Nepolean, T., Sankar, S. M., Singh, S. P., & Srivastava, R. K. (2017). Deciphering genomic regions for high grain iron and zinc content using association mapping in pearl millet. *Frontiers in Plant Science*, 8, 412.

Anuradha, N., Satyavathi, C. T., Bharadwaj, C., Sankar, M., & Pathy, L. (2018). Association of agronomic traits and micronutrients in pearl millet. *International Journal of Chemistry Studies*, 6, 181-184.

Anuradha, N., Satyavathi, C. T., Bharadwaj, C., Sankar, M., Singh, S. P. and Pathy T. L. (2018). Pearl millet genetic variability for grain yield and micronutrients in the arid zone of India. Journal of Pharmacognosy and Phytochemistry 7(1): 875-878

Anuradha, N., Satyavathi, C. T., Meena, M. C., Sankar, S. M., Bharadwaj, C., Bhat, J., & Singh, S. P. (2017). Evaluation of pearl millet [*Pennisetum glaucum* (L.) R. Br.] for grain iron and zinc content in different agro climatic zones of India. *Indian Journal of Genetics and Plant Breeding*, 77(1), 65-73.

Arora, P., Sehgal, S., & Kawatra, A. (2003). Content and HCl-extractability of minerals as affected by acid treatment of pearl millet. *Food Chemistry*, 80(1), 141-144.

Arulselvi, S., Mohanasundaram, K., Selvi, B., & Malarvizhi, P. (2007). Genetic variability studies and interrelationships among nutritional quality characters, phytate phosphorus

409 and grain yield in the seeds of pearl millet *Pennisetum glucum* (L.) R. Br. *Indian*
410 *Journal of Genetics and Plant Breeding*, 67(1), 37-40.

411 Azhaguvel, P., Hash, C. T., Rangasamy, P., & Sharma, A. (2003). Mapping the d 1 and d 2
412 dwarfing genes and the purple foliage color locus P in pearl millet. *Journal of*
413 *Heredity*, 94(2), 155-159.

414 Bhatt, J., Kumar, S., Patel, S., & Solanki, R. (2017). Sequence-related amplified polymorphism
415 (SRAP) markers based genetic diversity analysis of cumin genotypes. *Annals of*
416 *Agrarian Science*, 15(4), 434-438.

417 Bhattacharjee, R., Khairwal, I. S., Bramel, P. J., & Reddy, K. N. (2007) Establishment of a pearl
418 millet [*Pennisetum glaucum* (L.) R. Br.] core collection based on geographical
419 distribution and quantitative traits. *Euphytica*, 155:35-45.

420 Devos, K. M., Hanna, W. W. and Ozias-Akins, P. (2006). Pearl millet. In: C. Kole (eds):
421 Genome Mapping and Molecular Breeding in Plants, Volume 1, Cereals and Millets.
422 Springer–Verlag Berlin Heidelberg

423 Fasoula, D. A., Ioannides, I. M., & Omirou, M. (2020). Phenotyping and plant breeding:
424 overcoming the barriers. *Frontiers in plant science*, *10*, 1713.

425 Gabaza, M., Shumoy, H., Muchuweti, M., Vandamme, P., & Raes, K. (2018). Baobab fruit pulp
426 and mopane worm as potential functional ingredients to improve the iron and zinc
427 content and bio accessibility of fermented cereals. *Innovative Food Science &*
428 *Emerging Technologies*, 47, 390-398.

429 Glaszmann, J. C., Kilian, B., Upadhyaya, H. D., & Varshney, R. K. (2010). Accessing genetic
430 diversity for crop improvement. *Current opinion in plant biology*, *13*(2), 167-173.

431 Gopalan, C., Rama Sastri, B. V., & Balasubramanian, S. C. (2003). *Nutritive Value of Indian*
432 *Food*s. 156.

433 Govindaraj, M., Rai, K. N., Kanatti, A., Upadhyaya, H. D., Shivade, H., & Rao, A. S. (2020).
434 Exploring the genetic variability and diversity of pearl millet core collection germplasm
435 for grain nutritional traits improvement. *Scientific Reports*, 10(1), 1-13.

436 Govindaraj, M., Selvi, B., Rajarathinam, S., & Sumathi, P. (2011). Genetic variability and
437 heritability of grain yield components and grain mineral concentration in India's pearl
438 millet (*Pennisetum glaucum* (L) R. Br.) accessions. *African Journal of Food,*
439 *Agriculture, Nutrition and Development*, 11(3), 4758-4771.

440 Goyal, P., & Chugh, L. K. (2017). Shelf-life determinants and enzyme activities of pearl millet: a
441 comparison of changes in stored flour of hybrids, CMS lines, inbreds and
442 composites. *Journal of food science and technology*, 54(10), 3161-3169.

443 He, Q., Zhi, H., Tang, S. et al. QTL mapping for foxtail millet plant height in multi-environment
444 using an ultra-high density bin map. *Theoretical and Applied Genetics*, 134, 557–572
445 (2021).

446 Jackson, M. L. (1973). Soil chemical analysis, pentice hall of India Pvt. *Ltd., New Delhi,*
447 *India*, *498*, 151-154.

448 Kaur, K. D., Jha, A., Sabikhi, L., & Singh, A. K. (2014). Significance of coarse cereals in health
449 and nutrition: a review. *Journal of Food Science and Technology*, 51(8), 1429-1441.

450 Kaushik, I. & Grewal, R. B. (2017). Antinutrient and mineral content of thirteen different
451 varieties of pearl millet locally grown in Haryana, India. *International Journal of*
452 *Current Microbiology and Applied Sciences*, 6(5): 2136-2143.

453 Kumar, M., Rani, K., Ajay, B. C., Patel, M. S., Mungra, K. D., & Patel, M. P. (2020).
454 Multivariate diversity analysis for grain micronutrients concentration, yield and agro-
455 morphological traits in Pearl millet (*Pennisetum glaucum* (L) R. Br.). *International*
456 *Journal of Current Microbiology and Applied Sciences*, 9(3), 2209-2226.

457 Kumar, S., Hash, C. T., Thirunavukkarasu, N., Singh, G., Rajaram, V., Rathore, A., &
458 Srivastava, R. K. (2016). Mapping quantitative trait loci controlling high iron and zinc
459 content in self and open pollinated grains of pearl millet [*Pennisetum glaucum* (L.) R.
460 Br.]. *Frontiers in Plant Science*, 7, 1636.

461 Li, G., & Quiros, C. F. (2001). Sequence-related amplified polymorphism (SRAP), a new marker
462 system based on a simple PCR reaction: its application to mapping and gene tagging in
463 Brassica. *Theoretical and Applied Genetics*, 103(2), 455-461.

464 Liu, L. W., Zhao, L. P., Gong, Y. Q., Wang, M. X., Chen, L. M., Yang, J. L., & Wang, L. Z.
465 (2008). DNA fingerprinting and genetic diversity analysis of late-bolting radish
466 cultivars with RAPD, ISSR and SRAP markers. *Scientia Horticulturae*, 116(3), 240-
467 247.

468 Mace, E. S., Buhariwalla, K. K., Buhariwalla, H. K., & Crouch, J. H. (2003). A high-throughput
469 DNA extraction protocol for tropical molecular breeding programs. *Plant molecular*
470 *biology reporter*, *21*(4), 459-460.

471 Malik, C. P. and Singh, M. B . (1980). Plant enzymology and histo-enzymology. Food and
472 Agricultural Organization of the United Nations 286.

473 Maman, N., Mason, S. C., Lyon, D. J., and Dhungana, P. (2004). Yield components of pearl
474 millet and grain sorghum across environments in the Central Great Plains. *Crop*
475 *Science*, *44*(6), 2138-2145.

476 Maman, N., Mason, S., Lyon, D. J. and Dhungana, P. (2004). Yield components of pearlmillet
477 and grain sorghum across environments in the central great plains. *Panhandle Research*
478 *and Extension Centre*. Paper 3.

479 Marathe, A., Krishnan, V., Vinutha, T., Dahuja, A., Jolly, M., & Sachdev, A. (2018). Exploring
480 the role of Inositol 1, 3, 4-trisphosphate 5/6 kinase-2 (GmITPK2) as a dehydration and
481 salinity stress regulator in Glycine max (L.) Merr. through heterologous expression in
482 E. coli. *Plant Physiology and Biochemistry*, *123*, 331-341.

483 Nehra, M., Kumar, M., Vart, D., Kaushik, J., & Sharma, R. K. (2017). Molecular
484 characterization of pearl millet [*Pennisetum glaucum* (L.) R. Br] inbreds using
485 microsatellite markers. *Journal of Applied and Natural Science*, *9*(1), 357-363.

486 Oshodi, A. A., Oqungbenle, H. N. and Oladimeji, M. O. (1999). Chemical composition,
487 nutritionally valuable minerals and functional properties of benniseed (*Sesamum*

radiatum), pearl millet (*Pennisetum typoides*) and quinoa (*Chenopodium quinoa*) flours. *Int. J. Food Sci. Nutr*., 50: 325-331.

Pallavi, M., Reddy, P. S., Krishna, K. R., Ratnavathi, C. V., & Sujatha, P. (2020). Genetic variability, heritability and association of grain yield characters in pearl millet (*Pennisetum glaucum* L). *Journal of Pharmacognosy and Phytochemistry*, 9(3), 1666-1669.

Panse, V. G., & Sukhatme, P. V. (1978). *Statistical methods for agricultural worker*

Pujar, M., Gangaprasad, S., Govindaraj, M. and Kanatti, A. (2018).Genetic diversity analysis among advanced breeding lines in pearl millet for grain iron, zinc and agronomic traits. *In*: 1st National Genetics Congress on Genetics for Sustainable Food, Health and Nutrition Security, December 14-16, 2018, ICAR-lndian Agricultural Research Institute, New Delhi, India.

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

Pushparaj, F. S., & Urooj, A. (2014). Antioxidant activity in two pearl millet (*Pennisetum typhoideum*) cultivars as influenced by processing. *Antioxidants*, 3, 55-66.

Rai, K. N., Gupta, S. K., Sharma, R., Govindaraj, M., Rao, A. S., Shivade, H., & Bonamigo, L. A. (2014). Pearl millet breeding lines developed at ICRISAT: a reservoir of variability and useful source of non-target traits. *SAT eJournal*, *1*(1), 1-13.

Rai, K. N., Yadav, O. P., Govindaraj, M., Pfeiffer, W. H., Yadav, H. P., Rajpurohit, B. S., & Shivade, H. (2016). Grain iron and zinc densities in released and commercial cultivars of pearl millet (Pennisetum glaucum). *Indian Journal of Agricultural Sciences*, *86*(03), 11-16.

513 Ramya, A. R., Ahamed M, L., Satyavathi, C. T., Rathore, A., Katiyar, P., Raj, A. G., &
514 Srivastava, R. K. (2018). Towards defining heterotic gene pools in pearl millet
515 [*Pennisetum glaucum* (L.) R. Br.]. *Frontiers in Plant Science*, 8, 1934.

516 Rani S., T., Sameer Kumar, C. V., Maheswaramma, S., Parimal, M., Kumar, A., & Sravanthi, K.
517 (2019). Selection criteria for grain yield in pearl millet (*Pennisetum glaucam* L.) in
518 association with yield contributing traits. *International Journal of Pure & Applied*
519 *Bioscience*, *7*(3), 257-262.

520 Rohlf, F. J. (1998). NTSYS-pc: Microcomputer programs for numerical taxonomy and
521 multivariate analysis. *The American Statistician*, 41(4), 330-330.

522 Sharma, B., Chugh, L. K., Sheoran, R. K., Singh, V. K., & Sood, M. (2018). Study on genetic
523 variability, heritability and correlation in pearl millets germplasm. *Journal of*
524 *Pharmacognosy and Phytochemistry*, 7(6), 1983-1987.

525 Sharma, H. K., Sarkar, M., Choudhary, S. B., Kumar, A. A., Maruthi, R. T., Mitra, J., &
526 Karmakar, P. G. (2016). Diversity analysis based on agro-morphological traits and
527 microsatellite-based markers in global germplasm collections of roselle (*Hibiscus*
528 *sabdariffa* L.). *Industrial Crops and Products*, 89, 303-315.

529 Shobana, S., Sreerama, Y. N., & Malleshi, N. G. (2009). Composition and enzyme inhibitory
530 properties of finger millet (*Eleusine coracana* L.) seed coat phenolics: Mode of
531 inhibition of α-glucosidase and pancreatic amylase. *Food chemistry*, *115*(4), 1268-
532 1273.

533 Siles, M. M., Russell, W. K., Nelson, L. A., Baltengsperger, D. D., Johnson, B., Van, V. L. D.,
534 Jensen S. G. and Hein. G. L. (2004). Heterosis for grain yield and other agronomictraits
535 in foxtail millet. Faculty Papers and Publications in Animal Science. Paper 156.

536 Sokal, R. R. (1958). A statistical method for evaluating systematic relationships. *Univ. Kansas*,
537 *Sci. Bull.*, *38*, 1409-1438.

538 Sonali, S., Shikha, Y., Ramesh, K., Sushma, S., & Neeru, R. (2019). Multi trait analysis reveals
539 substantial diversity in pearl millet [*Pennisetum glaucum* (L.) R. Br.] inbred lines.
540 *Journal of Experimental Biology and Agricultural Sciences*, 7(4), 358-375.

541 Tomar, M., Bhardwaj, R., Kumar, M., Singh, S. and Pal, et al. (2021) Nutritional composition
542 patterns and application of multivariate analysis to evaluate indigenous Pearl millet
543 (*Pennisetum glaucum* (L.) R. Br.) germplasm. *Journal of Food Composition and*
544 *Analysis*, 103: 104086,

545 Upadhyaya, H. D., Reddy, K. N. and Gowda, C. L. L. (2007). Pearl millet germplasm at
546 ICRISAT genebank – status and impact. SAT eJournal, 3:1-5.

547 Velu, G., Rai, K. N., Muralidharan, V., Kulkarni, V. N., Longvah, T., & Raveendran, T. S.
548 (2007). Prospects of breeding biofortified pearl millet with high grain iron and zinc
549 content. *Plant Breeding*, 126(2), 182-185.

550 Vengadessan, V., Rai, K. N., Kannan Bapu, J. R., Hash, C. T., Bhattacharjee, R., Senthilvel, S.,
551 ... & Nepolean, T. (2013). Construction of genetic linkage map and QTL analysis of
552 sink-size traits in pearl millet (*Pennisetum glaucum*). *International Scholarly Research*
553 *Notices*, 2013(1):471632

554 Warrier, S. R., Patel, B. C., Kumar, S., & Sherasiya, S. A. (2020). Combining ability and
555 heterosis for grain minerals, grain weight and yield in pearl millet and SSR markers-
556 based diversity of lines and testers. *Journal of King Saud University-Science*, 32(2),
557 1536-1543.

558 Yadav, O. P., Gupta, S. K., Govindaraj, M., Sharma, R., Varshney, R. K., Srivastava, R. K., &
559 Mahala, R. S. (2021). Genetic gains in pearl millet in India: insights into historic
560 breeding strategies and future perspective. *Frontiers in Plant Science*, 396.

561 Yadav, O. P., Rai, K. N., Khairwal, I. S., Rajpurohit, B. S., & Mahala, R. S. (2011). Breeding
562 pearl millet for arid zone of north-western India: Constraints, opportunities and
563 approaches. *All India coordinated pearl millet improvement project, Jodhpur, India*, 28.

564 Yadav, O. P., Rai, K. N., Yadav, H. P., Rajpurohit, B. S., Gupta, S. K., Rathore, A. and Karjagi,
565 C. G. (2016). Assessment of diversity in commercial hybrids of pearl millet in India.
566 *Indian J. Plant Genet.Resour*., 29(2), 130-136.

567 Zala, H. N., Kulkarni, K. S., Bosamia, T. C., Shukla, Y. M., Kumar, S., Fougat, R. S., & Patel,
568 A. (2017). Development and validation of EST derived SSR markers with relevance to
569 downy mildew (*Sclerospora graminicola* Sacc.) resistance in pearl millet [*Pennisetum*
570 *glaucum* (L.) R. Br.]. *Journal of Plant Biochemistry and Biotechnology*, *26*(3), 356-
571 365.

572

573 Table 1. Analysis of variance (ANOVA) of morpho-biochemical traits in pearl millet

Trait	Source of variation and Mean squares		
	Replication (df = 1)	Genotypes (df = 47)	Error (df = 47)
Days to 50% flowering	0.167	322.311*	5.954
Plant height	66.833	917.408*	62.211
Head diameter	0.027	0.159*	0.027
Panicle length	1.927	46.955*	3.880
Productive tillers per plant	0.027	3.712*	0.217
Grain yield per plant	11.003	203.764*	30.441
Days to maturity	2.344	9.292*	3.471
1000 grain weight	0.000176	2.863*	0.104
Protein content	2.004	10.363*	0.705
Lipid content	0.062	3.851*	0.052
Iron content	14.015	276.360*	16.714
Zinc content	41.12	79.610*	13.100
Manganese content	2.154	11.232*	1.576
Calcium content	661.5	2061.205*	332.691
Copper content	0.218	43.735*	0.551
Potassium content	57.722	102.837*	14.517
Phosphorus content	5.782	12.004*	5.093
Phytate content	895.482	8396.470*	541.246
Total phenolic acid	26.471	112.792*	36.582

574 *Significant at 5% level of probability

575

576 Table 2. Mean performance of morpho-biochemical traits in pearl millet

Trait	Mean	Range	S. Em	CD @ 5%	CV%
Days to 50% flowering	54.69	34.00 (IC-370523) - 77.00 (ICMR 07999)	1.73	4.91	4.46
Plant height (cm)	149.42	110.10 (ICMR 06555) - 205.35 (IC-332715)	5.58	15.87	5.28
Head Diameter (cm)	1.53	1.03 (ICMR 10999) - 2.15 (ICMR 09333)	0.12	0.33	10.75
Panicle length (cm)	24.03	15.55 (ICMR 11888) - 38.05 (IC-332716)	1.39	3.96	8.2
Productive tillers per plant	4.80	2.65(ICMR 08222, ICMR 11999)-7.60(IC-370523)	0.33	0.94	9.7
Grain yield per plant	29.54	15.85 (ICMR 07222) - 56.75 (Nandi 75)	3.9	11.1	18.67
Days to maturity	85.64	80.50 (ICMR 12333) - 89.00(ICMR 08999, ICMR 07777)	5.82	16.57	9.71
1000 grain weight	7.14	4.93 (ICMR 06888) - 10.45 (ICMR 06555)	0.23	0.65	4.51
Protein content (%)	13.73	8.26(AICRP-PM-12)-20.06(ICMR 12555)	0.59	1.68	6.12
Lipid content (%)	4.68	2.72(ICMR 06999)-6.95(ICMR 08444)	0.16	0.46	4.89
Iron content (ppm)	49.69	31.58 (ICMR 07777)-77.38 (ICMR 08666)	2.89	8.22	8.23
Zinc content (ppm)	39.36	29.34 (ICMR 08111)-55.48 (IC 139903)	2.65	7.53	9.5
Manganese content (ppm)	14.04	7.20 (ICMR 07222)-17.63(ICMR 08444)	0.89	2.53	8.94
Calcium content (ppm)	199.31	100.00 (ICMR 10222)–256.00 (ICMR 12888)	12.9	36.69	9.15
Copper content (ppm)	9.86	4.92 (AICRP-PM- 6)-22.59(GHB 558)	0.52	1.49	7.52
Potassium content (ppm)	4798	1800 (ICMR 07222)-6020(ICMR 10999)	2.69	7.67	7.94
Phosphorus content (ppm)	3112	2258(AICRP-PM-62)-3672(ICMR 08666)	1.6	4.54	7.25
Phytate content (mg/100g)	282.39	201.5(ICMR 08111)-542.5(GHB 558)	16.45	46.8	8.24
Total phenolic acid (mg/100g)	60.26	75.16(ICMR 12555)-44.41(Nandi 75)	4.28	12.17	10.04

577

578 Table 3. Top eight genotypes for different nutritional traits with their agronomic performance

Trait	Top 8 genotypes	Range	
		DFF	TGW (g)
Fe	ICMR 08666, ICMR 11888, IC 332727, IC 139900, ICMR 12555, ICMR 06111, ICMR 08333, ICMR 07888	37.00-72.00	5.91-8.45
Zn	IC 139903, ICMR 08999, ICMR 06222, ICMR 12999, ICMR 10999, ICMR 08666, IC 332715, IC 332703	42.00-70.50	5.94-9.39
Mn	ICMR 08444, ICMR 12555, ICMR 09888, ICMR 08333, ICMR 06666, ICMR 12666, ICMR 09222, IC 332703	45.00-74.50	5.91-8.01
Ca	ICMR 12888, ICMR 12777, ICMR 07444, ICMR 09333, ICMR 11777, IC 332703, ICMR 08999, ICMR 08333	35.50-72.50	5.91-8.36
Cu	GHB 558, IC 332715, IC 332716, ICMR 06666, ICMR 07222, ICMR 07444, ICMR 07777, ICMR 10999	42.00-73.50	5.71-8.02
K	GHB 558, GHB 732, GHB 905, ICMR 08666, ICMR 10999, ICMR 12555, ICMR 12666, ICMR 12777	43.50-74.00	6.16-8.99
P	ICMR 08666, ICMR 06888, ICMR 06111, IC 370523, IC 332727, IC 139899, Nandi 75, ICMR 06666	34.00-73.50	4.93-9.34

579 DFF: days to 50% flowering; TGW: 1000-grain weight

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581 Table 4.Variability for mean values of 19 quantitative traits in nine groups identified by Manhattan dissimilarity coefficient

	Number of genotypes in each cluster								
Trait	7	25	4	4	3	2	1	1	1
Days to 50% flowering	72.86	50.24	50.50	44.88	57.67	62.00	53.50	56.50	70.50
Plant height (cm)	126.56	152.87	174.28	139.36	172.68	139.78	125.45	135.95	151.25
Head Diameter (cm)	1.66	1.55	1.49	1.59	1.41	1.16	1.65	1.45	1.03
Panicle length (cm)	23.71	23.68	25.99	23.90	30.53	19.23	22.00	26.00	17.95
Productive tillers per plant	3.67	4.98	4.36	5.29	4.70	6.70	5.90	4.70	3.50
Grain yield per plant	24.72	29.63	48.99	22.53	27.23	22.33	47.75	15.85	28.35
Days to maturity	86.50	85.12	86.13	88.38	87.00	81.50	85.00	86.00	84.00
1000 grain weight	7.83	7.12	7.79	6.50	7.04	6.32	7.27	5.71	6.16
Protein content (%)	12.50	14.49	12.72	12.61	9.71	13.69	20.06	15.72	15.71
Lipid content (%)	5.67	4.36	4.72	5.64	3.25	4.13	2.82	6.92	6.64
Iron content (ppm)	52.03	50.39	37.14	55.66	46.22	43.33	69.65	48.98	46.16
Zinc content (ppm)	36.59	40.25	35.21	47.05	36.07	30.54	35.13	44.98	48.79
Manganese content (ppm)	13.63	14.94	13.65	13.30	14.40	9.66	17.28	7.20	10.39
Calcium content (ppm)	191.57	208.04	144.75	207.25	215.83	158.75	229.00	204.50	218.50
Copper content (ppm)	9.40	8.02	7.19	13.85	19.78	9.44	8.18	17.29	19.29
Potassium content (ppm)	45.70	49.33	46.08	49.31	49.00	45.58	52.30	18.00	60.15
Phosphorus content (ppm)	31.43	31.81	30.68	27.54	31.98	29.23	27.75	31.52	32.07
Phytate content (mg/100g)	283.74	272.46	327.68	248.10	398.32	206.55	287.75	307.10	250.95
Total phenolic acid (mg/100g)	58.59	58.50	58.21	66.53	65.50	59.57	75.16	56.96	73.15

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586 Table 5. Amplification details of DNA markers

SSR marker system							
Maker name	Band size (bp)	TB	Polymorphism (%)	PI	PIC	RP	Mean RP
PMES153	145-154	4	100	1.14	0.29	2.00	0.50
PMES157	135-151	4	100	1.46	0.37	1.96	0.49
PMES160	134-150	4	100	1.36	0.34	2.13	0.53
PMES161	155-233	3	100	1.23	0.41	2.04	0.68
PMES162	132-162	7	100	1.35	0.19	1.63	0.23
PMES168	222-284	10	100	1.96	0.19	2.38	0.24
PMES170	154-195	9	100	1.43	0.16	2.00	0.22
PMES171	151-292	13	100	1.71	0.13	2.00	0.15
PMES173	216-203	8	100	0.99	0.50	2.00	1.00
PMES185	176-200	8	100	1.55	0.19	1.83	0.23
PMES190	85-104	6	100	1.53	0.26	1.92	0.32
PMES199	213-305	4	100	1.27	0.32	1.83	0.46
Average		6.17		1.42	0.28	1.98	0.42
SRAP marker system							
Em6+Me2	205-1234	25	100	8.11	0.32	13.25	0.53
Em2+Me2	94-1152	34	100	7.96	0.26	10.20	0.30
Em1+Me2	223-1125	18	100	5.33	0.30	6.88	0.38
Em5+Me4	120-1020	17	100	3.81	0.22	4.63	0.27
Em6+Me3	450-1357	10	100	2.33	0.23	5.42	0.54
Em2+Me3	313-1065	26	100	6.57	0.25	9.25	0.36
Average		21.67		5.69	0.26	8.27	0.4

587 TB: Total Number of Bands; PI: Primer index; PIC: Polymorphic Information Content; RP: Resolving Power.
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589 Table 6. Comparison between SSR and SRAP marker system

Marker system	Total markers	TB	PB	FP	H_{av}	MR	EMR	MI
SSR	12	74.00	74.00	1.00	3.35	5.69	5.69	19.092
SRAP	6	130.00	130.00	1.00	1.57	10.00	10.00	15.655

590 TB: Total Number of Bands; PB: Number Polymorphic Bands; PI: Primer index; PIC: PolymorphicInformation Content; RP: Resolving Power;
591 FP: Fractionation of Polymorphism; Hav: Average PIC; MR: Multiplex Ratio; EMR: Effective Multiplex Ratio; MI: Marker Index.

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593 **Fig 1. Dendrogram based on genetic coefficient of similarity among pearl millet genotypes**