Morpho-biochemical characterization and molecular ~~marker~~ marker-based genetic 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 was~~ere~~ evaluated for eight morphological traits and eleven biochemical characters, and genetic diversity was performed~~. Genotypes were characterized~~ using twelve SSR and six SRAP markers. ~~Significant~~ The significant mean difference ~~among~~ between morphological and biochemical ~~was~~ traits w~~ere~~as detected in ANOVA. The productive tillers per plant varied from 2.65 to 7.60 with a mean of 4.80. The ~~G~~ 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 content ~~was~~ w~~ere~~as 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~~ superior for Fe, Zn, K, and P. The inter-genotype similarity coefficient at the genetic level, generated using DNA

Açıklamalı [Ref1]: Instead of "study in" the word "of" would be better.

Açıklamalı [Ref2]: Sentence is too long, can be divided in to two sentences.

Açıklamalı [Ref3]: There is no need to write ANOVA.

Açıklamalı [Ref4]: Can be improved for writing quality and may be written shorter. Some of the "the" are not relevant.

markers, ranged from 0.616 to 0.877 with a mean of 0.743. A ~~C~~ combination of ~~phenotypic~~ morpho-biochemical traits~~–~~ and ~~genotypic~~ DNA markers~~–~~-based diversity ~~carried out in this research~~ will help to differentiate the genotypes and diverse genotypes can be used in the breeding programs to improve the mineral content in pearl millet. ~~under study in a better way.~~

***Key-words*:** Genetic ~~D~~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~~ ~~in~~ drought, nutrient-depleted soil, and ~~high temperature~~ hot environmental conditions of the ~~hot~~ hostile deserts of India~~n~~ and Africa~~n~~. This hardy nature makes pearl millet ~~a~~ resilient to harsher climatic conditions consequently it is cultivated in marginal environments of arid and semi-arid tropical regions of India and ~~s~~South~~–~~east Asia, sub-Saharan Africa, and much of southern and eastern Africa (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~~ may allow the improvement of micronutrient~~s~~ density in grain and grain yield.

Açıklamalı [Ref5]: may

Açıklamalı [Ref6]: while importance of pearl millet is given quite excessively. Similar genetic diversity studies are not given.

Açıklamalı [Ref7]: should be in a single paranthesis, not two.

Micronutrient insufficiency has emerged as a global problem, particularly for those living in underdeveloped nations and consuming carbohydrate-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 iron (Fe) and zinc (Zn) concentration and essential amino acids. In 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 grains of pearl millet areare gluten-free and have a low glycemic index due to their grains have high fibreer content 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 extentd only. Moreover, the presence of anti-nutrients factors like saponins, tannins, and phytic acid which can reduce nutrient utilization or food uptake 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 genotypes would have different phenotypes due to environmental variabilitysimilar 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) markers for genetic diversity assessment though SRAP has been used for linkage map development by Pedraza-Garcia et al. (2010). Therefore, in the current experiment, both SRAP and microsatellite markers have been used to expose the genetic diversity. SRAP

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Açıklamalı [Ref9]: Wheat has a wide range in protein content. https://www.mdpi.com/1422-0067/12/9/5878

So, writing 11,8 would cause misunderstanding.

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Açıklamalı [Ref11]: Not found in the references.

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-nutrients content in grain along with molecular diversity in pearl millet germplasm.

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2. Materials and methods

With two replications, tThe field trial was done in a randomized complete block design (RCBD) 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,The panicles were covered with glassine bags to prevent cross-pollination by outside pollen and to collect self-seeds. For phenotyping of grain--based 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 PD 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 (Anton Paar GmbH, Austria). 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 10 mins ~~on~~ at 70°C temperature and a holding time of 5 min~~utes~~. 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 rate in a peristaltic ~~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 simple sequence repeat (SSR) and sequence-related amplified polymorphism (SRAP) markers. For microsatellite marker profiling, markers from the PMES series (Zala et al., 2017) were amplified in SensoQuest Thermocycler (Germany). The SSR-PCR reaction conditions were as follows: 94°C (initial denaturation) for 5 mins., 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 mins (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)~~.~~ An agarose gel (3%) was used to resolve PCR products.

2.4. Statistical analysis

Açıklamalı [Ref13]: Please either use microsatellite or SSR.

147 The mean value of traits was figured out, and analysis of variance (ANOVA) was performed in
148 accordance with Panse and Sukhatme (1978) in Microsoft Excel 2013. A ~~P~~ phenotypic trait-
149 based dissimilarity matrix was constructed using Manhattan coefficients with Numerical
150 Taxonomy and Multivariate Analysis System (NTSYSpc 2.0; ~~(~~Rohlf 1998). The amplified
151 products of SSR and SRAP markers were scored in 1 (presence) and 0 (absence) fashion.
152 Polymorphism information content (PIC), Multiplex ratio (MR), effective multiplex ratio (EMR)
153 marker index (MI), and resolving power (Rp) value were estimated following Sharma et al.
154 (2016) in Microsoft Excel 2013. In NTSYSpc 2.0, The SIMQUAL program used ~~the~~ Jaccard's
155 similarity (J) coefficient to compute the genetic similarity between genotypes, SAHN clustering
156 method was used to construct the unweighted pair group method with arithmetic mean
157 (UPGMA) dendrogram (Sneath and Sokal, 1973).

158 **3. Results and discussion**

159 ~~*3.1. ANOVA*~~

Biçimlendirilmiş: Girinti: Sol: 0.75 cm, Madde işaretleri veya numaralandırma yok

160 ~~Genetic variability, a quantitative measure of genetic dissimilarities, is the total of genetic~~
161 ~~diversity within a population. The genetic differences among genotypes are the result of natural~~
162 ~~selection over a period of time. Greater variability present in the base breeding germplasm~~
163 ~~ensures good opportunities to produce desired genetic architecture of a plant.~~ The ANOVA
164 resulted that genotypic variations were ~~very~~ significant at a 5% level of probability for all the
165 traits, showing ~~an~~ ample genetic diversity among the genotypes under study~~.~~ (Table 1). This also
166 suggested that there is sufficient scope to select superior breeding material which can be
167 exploited in pearl millet breeding programs.

168 ~~*3.2.*~~*3.1.* *Character variance analysis*

169 ~~*3.2.1.*~~*3.1.1.* *Morphological parameters*

170 Early flowering is a desirable trait for pearl millet as it is a crop of ~~(~~semi~~)~~-arid and arid regions.
171 Earliness becomes an important trait in areas where scanty and erratic rains aggravate the
172 moisture stress condition during the growth stage of the crop and leads to ~~a~~ post-flowering
173 moisture stress (Yadav et al., 2011). In the current study, though, the population mean for days
174 to 50% flowering was 54.69 days but the days to 50% flowering ranged from 34 (IC 370523) to
175 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. 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 ~~-~~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, a complex trait, is one of the supreme traits which is influenced by several associated traits. ~~This makes grain yield a complicated trait.~~ The ~~G~~ 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 (TGW) 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

Açıklamalı [Ref14]: related?

Açıklamalı [Ref15]: In the methods it was in mm, here it is given as cm.

Açıklamalı [Ref16]: The term "bold seed" is not explained in the manuscript. It is not enough to define it in the referee response letter.

Açıklamalı [Ref17]: have

revealed huge variability for ~~1000 seed weight~~ the TGW (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.~~*3.1.2. Biochemical parameters*

Compared ~~ing to~~ to other main cereal crops, pearl millet yields more nutritious grains ~~that are a great source of~~ with 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 (parts per million), 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 39.36 (Table 2). A similar mean value was ~~ere~~ 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).

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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 regulation ~~g~~ heart beats 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 ppm 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 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 (3667-5133 ppm; Govindaraj et al. 2020b).

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%. 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

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by hydrothermal treatment, irradiation, cooling storage, or a combination of more than one technology. (Goyal and Chugh, 2017)

The metal--chelating ability of phytic acid makes it ~~is~~ an antinutritional phytochemical 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. Abdalla et al. (2007) also recorded ~~A~~ a similar range from 354-795 mg/100g of phytate ~~was detected by Abdalla et al. (2007~~where ~~; 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 in pearl millet grain is significantly lower than in rice (0.68-1.03%; Liu, 2005), oat (0.5–1.2%; Peterson, 2001), ~~soybean~~ soybean (1.0–2.22%; Lolas et al. 1976) and wheat (0.2 - 2.9%; Gupta et al. 2015)~~.~~ Hence, regular consumption will possibly not hamper the bioavailability of minerals.

Açıklamalı [Ref20]: Reference?

Polyphenols have many health benefits as having antioxidant activity. Moreover, phytic acid is considered to be beneficial in dropping cholesterol and ~~reduces~~ reduceing cancer risk. The values of total phenolic acid ranged ~~with~~ from 75.16mg/100g (ICMR 12555) to 44.41mg/100g (Nandi 75) with 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).

Açıklamalı [Ref21]: When we use the sentence style "from.....to...." it is generally written from the smaller number to larger one.

~~3.3.~~3.2. *Nutrient-dense genotypes*

Genotypes dense in multiple nutrients can directly be released as a variety after evaluating ~~on 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.~~3.3. *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 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 five~~ 25 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~~ TGW. 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 low values 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 grain weight~~ TGW (Table 4).

~~3.5.~~3.4. *Molecular marker~~–~~based diversity~~study~~*

~~During recent days molecular markers have been commonly using for assessing genetic diversity. The combined use of two or more markers (both dominant and codominant) for genetic~~

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Açıklamalı [Ref23]: Material and methods not here.

Açıklamalı [Ref24]: Not capitalized.

~~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). During the experiment, a total of 50 SSR markers were screened for amplification. Out of 50 ~~SSR~~markers, 32 (60%) primers showed amplification. Of these 30 SSR markers, 12 (37.5%) markers were found~~, of 30 SSR primers certified~~ polymorphic. These 12 polymorphic SSRs markers generated 65 ~~alleles~~amplicons. The molecular weight of the amplicon ~~with band sizes ranging~~ranged from 85bp (PMES 190) to 292bp (PMES 171). In previous reports with PMES-series SSR markers, ~~Earlier~~, Zala et al. (2017) recorded amplicon size from 101 to 285 bp ~~was reported by Zala et al. (2017)~~. The number of polymorphic bands/amplicons per SSR marker ranged from 2 to 13, with a mean of 6.71. ~~Zala et al. (2017) reported 2.20 alleles.~~ All SSR amplicons were found polymorphic ~~The percentage polymorphism is 100%~~. The PIC value, the informativeness of a primer, for each marker was computed for the estimation of marker allelic variation ~~based on~~ considering the allele frequencies in studied genotypes. ~~PIC designates the informativeness of a primer.~~ The mean PIC of SSR markers was 0.28 ~~on average~~ though it ~~and~~ varied from 0.132 (PMES 171) to 0.499 (PMES 173). This range was comparable with Zala et al. (2017) where PIC was between ~~;~~ 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 the 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 the 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 in cumin. This suggested that in different crops SRAP amplicon size will be highly variable. 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 markers 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 The fraction of polymorphism, MR, EMR and MI for SRAPs are 1.00, 10.00, 10.00, and 15.65, respectively (Table 6).

3.6.3.5. *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 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.

Açıklamalı [Ref25]: Genomic or genetic?

4. Conclusion

The genetic diversity for morphological and grain biochemical traits, an outcome of natural selection with the 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 namely 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 share~~ had only 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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603 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

604 *Significant at 5% level of probability

605

Açıklamalı [Ref26]: Phenological trait

Açıklamalı [Ref27]: Yield trait

Açıklamalı [Ref28]: Yield component

606 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

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Açıklamalı [Ref29]: What is S.Em?

Açıklamalı [Ref30]: All abbreviations used in a given table should be explained in full below the table.

608 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

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

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Table 4. Variability for mean values of 19 quantitative traits in nine groups identified by the 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

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

TB: Total Number of Bands; PI: Primer index; PIC: Polymorphic Information Content; RP: Resolving Power.

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

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

Açıklamalı [Ref31]: marker

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