

Phenotypic Variation and Genotypic Diversity of Finger Millet (*Eleusine coracana* L. Gaertn) Germplasm Collections in Malawi.

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Abstract

Finger millet (*Eleusine coracana* L. Gaertn) has remained neglected and underutilized for years in Malawi partly due to limited research that include crop improvement. Any effort to exploit the genetic potential of the available genetic variability would lead to identification of potential germplasm for production in Malawi. The study aimed at characterizing agro-morphological traits and identifying genetic diversity using Kompetitive Allele Specific PCR (KASP) single nucleotide polymorphism (SNP) markers in order to identify unique traits that can be used in germplasm discrimination for seed production as well as crop improvement. A total of 40 finger millet germplasm collections were evaluated in the field at Crops and Soil Sciences Department's research farm of Lilongwe University of Agriculture and Natural Resources (LUANAR). The experiment was laid out in an Alpha lattice design. From 40 germplasm collections, 188 germplasm collection samples were selected for Deoxyribonucleic Acid (DNA) extraction. Phenotypic data on agro-morphological traits was collected and subjected to multivariate analysis. Genotypic data was subjected to Analysis of Molecular Variance (AMOVA) in R- statistical package. Three principal components explained 42% of the total phenotypic variation among the germplasm collections while AMOVA revealed a 48% variation within and 53% variation among the germplasm collections. Cluster analysis grouped the 188 genotyped germplasm collections into 6 clusters. Phenotypic variations and genotypic diversity exist in finger millet which aids in the discrimination of the germplasm collections in seed production and in breeding programs.

Introduction

Finger millet (*Eleusine coracana* L. Gaertn) is a cereal crop used for food, fodder, and industrial crop grown in several Asian and African countries (Bharathi, 2020, Ojulong et al., 2021). It is an annual allotetraploid ($2n = 4x = 36$) crop that is highly self-fertilized (Sood et al., 2016). The nutritional properties of finger millet have been studied and reported to be the best among all cereals (Gull et al., 2014, Gautam et al., 2021, Barao et al., 2022). Finger millet grain has a carbohydrate content of 65–75%, protein content of 6–13%, crude fiber content of 3.6%, and mineral content of 2.7% when compared to other cereal crops such as rice, which has a carbohydrate content of 10% and protein content of 5% (Chandra et al., 2016, Gull et al., 2014, Gautam et al., 2021, Barao et al., 2022). Its protein profile is relatively well balanced with more lysine, threonine, and valine than other millets. It was also reported by Chandra et al., (2016) that the crop is high in calcium ($220\text{--}450\text{ mgkg}^{-1}$) and iron ($3\text{--}20\text{ mgkg}^{-1}$). Chandra et al., (2016) also reported that finger millet contains several amino acids that are lacking in many cereals, including isoleucine (4.4 g), leucine (9.5 g), methionine (3.1 g), and phenyl alanine (5.2 g). Finger millets are also high in B vitamins, particularly niacin, B6, and folic acid. Despite its nutritional benefits and adaptation to marginalized environments, the crop has remained neglected and underutilized in Malawi.

The value of finger millet for farmers include culture and food tradition which contributes to food and nutritional security, circular and solidarity economy which contributes to income from seed and grain, recycling through livestock feed and soil structure improvement when incorporated in the soil (Turner, 2018, Yadav et al., 2018). Little is known about the agromorphological characteristics of finger millet

which is essential for seed characterization and identification of seed purity (Goswami et al., 2015). Agromorphological characterization together with molecular characterization using molecular markers will help to reveal the genetic diversity that exists among finger millet germplasm collections and these can be used by farmers in selecting best performing germplasm collections and by breeders for crop improvement programs (Umar & Kwon-ndung, 2015). Currently there is low production of finger millet in Malawi as shown in Table S4 partly due to lack of improved varieties (MoAFS, 2020). Variations within individual gene loci/among alleles of a gene, or gene combinations, between individual plants or plant populations are referred to as genetic diversity (Sood et al., 2016). Since finger millet is grown in a variety of climatic conditions in Malawi, genetic diversity analysis aids in understanding genome variation between genotypes and subsequent population development for molecular marker analysis (Ceasar et al., 2018). The genotyping of finger millet germplasm collections provides information that can be used to improve plant genetics resource management in a variety of ways. Knowledge of genetic diversity is required to properly manage and use finger millet landraces and germplasm (Joshi et al., 2020). Most studies on diversity analysis using molecular markers have found a low level of polymorphism in cultivated finger millet. This could be due to the limited genetic pool of cultivated finger millet (Sood et al., 2016).

A number of molecular markers have been used to identify genetic relationships among finger millet genotypes and these play an important role in germplasm characterization. Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR) or Microsatellite Assay, and Amplified Fragment Length Polymorphism (AFLP) are some of the molecular markers used to identify genetic relationships (Goron & Raizada, 2015). Another study reported the use of Simple Sequence Repeats (SSR) and Single Nucleotide Polymorphism (SNP) at the same time. A few polymorphic markers were discovered in cultivated finger millet (Gimode et al., 2016). According to Gimode et al., (2016), the 80 polymorphic SNP markers developed in the study successfully discriminated between different finger millet species and allowed for the correct classification of unknown species. Currently there is lack of good quality seed for finger millet in many countries since there are few improved varieties that have been developed in addition to local varieties used by farmers (Owere et al., 2014, Gebreyohannes et al., 2021). Farmers who grow finger millet in Malawi rely on seed they have saved or exchanged with other farmers. Exchange is only possible when farmers have a surplus of seeds to offer, which is not always the case (Andersen et al., 2022). Strategies for increasing crop productivity are the generation, popularization, evaluation, and dissemination of finger millet germplasm with full package management technologies and farmer involvement (Chemada et al., 2021). Farmer-led seed enterprise can lead to production and marketing of high-quality fair priced seed at the same time promoting income security among smallholder farmers. However, for them to thrive, a conducive policy and regulatory framework that is inclusive of smallholder farmers' engagement and less stringent is required. Quality Declared Seed (QDS) are seeds produced by organized and registered smallholder farmers in conformity with the required quality standards (Mastenbroek et al., 2021). The QDS production and marketing system is intended to create a more hospitable environment for farmer-led seed enterprises (Mastenbroek et al., 2021).

Therefore, this research aimed at characterizing finger millet's agro-morphological traits to assist in identification of unique traits that can be used for selection of quality finger millet germplasm by smallholder farmers and that can be promoted for seed production in Malawi. The research also aimed at identifying genetic diversity of finger millet germplasm using Kompetitive Allele Specific PCR (KASP) single nucleotide polymorphism (SNP) markers. The information will help plant breeders who want to embark on crop improvement program. Seed access in Malawi can be achieved through proper seed characterization in collaboration with farmers and this can only be done through farmer managed seed systems. This approach is expected to lead to the improvement of finger millet seed and improved adoption by farmers since they will learn to produce and identify pure seed (Owere et al., 2014). This will also help to ensure availability of quality declared seed produced by farmers through integrated seed systems. This type of seed systems falls between an informal and formal seed systems (Mastenbroek et al., 2021, Almekinders & Louwaars, 2002).

Materials And Methods

Germplasm collections sites

Forty finger millet germplasm collections were collected in Eswazini, Chipala, Kandeu, Manjawira and Mombezi Extension Planning Area (EPA) as shown in Figure S1. Germplasm collections were identified at seed fairs from farmer saved seeds with complete description of their attributes. Samples of some germplasm collections were provided by the National Plant Genetic Resources Centre (NPGRC) and International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) Malawi.

Experimental design and field layout

The field experiment was carried out during the 2020/2021 growing season at the Department of Crops and Soil Sciences Research Farm of the Lilongwe University of Agriculture and Natural Resources (LUANAR). The farm is located at an elevation of 1158 m above sea level with a latitude of 14°35'S and a longitude of 33° 50'E. The average annual temperature ranges from 18°C to 27°C and the area receives between 800 mm to 1031 mm of rain per year during the months of December to April. The experimental set up was Alpha lattice with three replications. The seeds were planted in 3 m long ridges with 0.75 m spacing between ridges and 10 cm between plants with one seedling per planting station. Weeding, banking, fertilizer application, pest and disease management were monitored and managed.

Phenotypic data collection and analysis

Qualitative and quantitative data on all agro-morphological traits of finger millet were collected beginning at the vegetative, germination to harvesting and seed processing stage following International Board for Plant Genetic Resources (IBPGR, 1985) finger millet descriptor and subjected to multivariate analysis. Principal component analysis and biplot of principal component were generated in R-statistical package. Excel were used to develop graphs for qualitative data.

Leaf sample collection for DNA extraction

A total of 188 samples selected from the 40 germplasm collections were planted in the year 2021 in the screen house at LUANAR. The plants were allowed to grow for three weeks to allow full establishment. A single-hole puncher was used to collect the leaf disc from a fresh clean leaf and a pair of forceps was used to place the two leaf discs in the wells of the 96 well plate until the 94 wells were filled. DNA extraction was done using sbeadex™ DNA extraction kit from LGC, Bioserach Technologies. Twenty - three Kompetitive Allele Specific PCR (KASP) SNP markers for Finger millet were used to genotype the germplasm collections.

Analysis of genotype data

Analysis of genotype data was done in the R software environment (R Core Team, 2021). Assessment of the SNPs for quality was done using the snpReady package (Granato & Fritsche-Neto, 2018) and entailed filtering for minor allele frequency (MAF), call rate and heterozygosity at thresholds of 0.01, 0.3 and 0.3 respectively. Principle component analysis (PCA) was done using the factoextra package (Kassambara and Mundt, 2016). A euclidian distance matrix was generated using the dist function in R. This was used for hierarchical clustering and dendrogram plotting. GeneALEX v 6.5 (Peakall & Smouse, 2012) was used for AMOVA with population groupings deduced from dendrogram clustering.

Results

Qualitative trait variation

Qualitative traits were evaluated visually for the 40 finger millet germplasm collections. The most common plant colour variation was lack of pigmentation accounting for 77.5% of the germplasm collections while pigmented germplasm collections accounted for 22.5% as shown in Figure 1a. Prostrate growth habits accounted for 82.5 % of the germplasm collections while 17 % exhibited erect growth habits and 0.5% exhibited decumbent growth habit as shown in Figure 1b. Semi-compact ears accounted for 52.5 % of the germplasm collections, with open ears accounting for 37.5% and compact and fist-like ears accounting for 5% as shown in Figure 1c. There was variation in seed colour among the germplasm collections that were evaluated where 50% had a light brown colour, 45% had copper brown colour 2.5% had white color and purple brown color respectively as shown in Figure 1d.

Principal Component Analysis (PCA) of the 14 agro-morphological traits

The qualitative traits were examined to determine which traits account for the majority of the variation between germplasm collections. Eigen values were used to determine the main axis of the data analyzed because the main axis, also known as the first principal component, is the axis in which the data varies the most. Therefore, the three components with eigenvalues equal to or greater than one were kept. PCA revealed a total variation of 42% between the germplasm collections as shown in table 2. The first principal component (PC1) has an Eigen value of 3.272 and represents 19% variation, representing the

equivalent of four individual variables. The four variables that weighted higher and contributed positively to variation are peduncle length (cm), number of fingers per plant, flag leaf blade length (mm), and yield (kg ha^{-1}). The second principal component (PC2) has an Eigen value of 2.167 and represents 12% of total variation, representing the equivalent of three individual variables. The three variables that weighted higher and contributed positively to variation are number of culm branches, leaf sheath length (cm), leaf sheath width (mm), and leaf blade length (cm). The third principal component (PC3) with an Eigen value of 2.019 and reflects 11% variations exhibited significant positive effects for peduncle length (cm), finger width (mm), number of days to flowering, and leaf sheath width (cm) making it the major contributor to the significant germplasm variations for agronomic traits among the finger millet germplasm collections.

Table 2: Principal components analysis of the 14 agro-morphological quantitative traits for 40 finger millet germplasm collections evaluated at Bunda during 2020/2021 growing season.

Principal components (PC)	PC1	PC2	PC3
Eigen values	3.272	2.167	2.019
Proportion of Variance %	19	12	11
Cumulative Proportion %	19.3	32	43.9
Number of days to flowering	-0.129	-0.391	0.38
Plant height (cm)	-0.154	-0.487	0.142
Finger length (cm)	-0.262	-0.187	0.288
Finger width (mm)	-0.186	0.145	0.353
Peduncle length (cm)	0.301	-0.274	0.301
Number of fingers per plant	0.405	-0.29	-0.213
Number of leaves per plant	-0.297	-0.255	-0.166
Leaf sheath length (cm)	-0.405	0.278	-0.339
Leaf sheath width (mm)	-0.265	0.234	0.333
Leaf blade length (cm)	-0.313	0.22	0.132
Leaf blade width (mm)	-0.325	-0.2	0.128
Blade length of flag leaf (cm)	0.342	-0.375	-0.438
Harvest index	0.329	0.124	0.17
Yield Kgha^{-1}	0.409	0.229	0.164

Biplot of principal component analysis based on 15 agro-morphological quantitative traits

The bi-plot depicts the distribution of germplasm collections based on different Principal component (PC) scores (PC1, PC2, and PC3), as well as the relationship between different traits and PC scores. A biplot of the 40 finger millet germplasm collections evaluated at Bunda during the 2020/2021 growing season is shown in Figure 2. Based on Principal Component (PC) 1 and 2, yield (kg ha^{-1}), number of fingers per plant, leaf blade length (cm), leaf sheath length (cm), number of leaves per plant, plant height (cm), and peduncle length (cm) had relatively long vectors, indicating that germplasm collections varied widely. These traits can be the standard features used to determine similarities and diversity of species phenotypes. In other words, they show large variations across the 40 germplasm collections studied, implying that they are the best discriminators of the morphological traits under consideration. Number of days to flowering, flag leaf of blade length (cm), and flag leaf of blade width (mm), on the other hand, had the shortest vectors, indicating that they are the least discriminator of the morphological traits under study. The biplot of principal component analysis categorized the clusters based on the characteristics of the germplasm collections. Cluster I was classified as having high yielding (kg ha^{-1}), low leaf sheath width (mm) and more number of leaves. Cluster II was classified as having low yield (kg ha^{-1}) and shorter flowering time, whereas Cluster III was classified as having high yield (kg ha^{-1}) and low number of leaves. Cluster IV was classified as having low yield (kg ha^{-1}), low biomass (kg ha^{-1}), more number of fingers per plant, short plant height (cm) and longer flowering time.

SNP characteristics

In this research 23 KASP SNP markers were used. Out of the 23 SNPs, only 18 were retained. The SNP markers had an average Minor allele frequency (MAF) of 0.15, Neis genetic diversity (GD) of 0.2, observed heterozygosity (H_o) of 0.11, polymorphic information content (PIC) of 0.16 and an inbreeding coefficient (F_i) of 0.51 as shown in Figure S2.

Only five of the 23 SNPs tested were found to have a high ability to capture variation and these are snpEC00201, snpEC00223, snpEC00222, snpEC00218 and snpEC00220 as shown in Figure S3. The 5 SNPs can effectively reveal genetic diversity in finger millet germplasm collections. Whereas snpEC00215, snpEC00199, and snpEC00225 were identified to have a low ability to capture variation and these cannot be used in the identification of genetic diversity among and between the germplasm collections.

Cluster analysis of the 188 finger millet germplasm collection samples genotyped

Genotypic data grouped the 188 germplasm collection samples into 6 clusters as shown in figure 6. The largest cluster had 61 germplasm collections while the smallest cluster had 10 germplasm collections. Cluster 1 had most of the germplasm collections from Kandeu EPA, cluster 2 had most of the germplasm collections from ICRISAT while cluster 3, 4, 5 and 6 had most of the germplasm collections from Genebank and Mombezi EPA.

Analysis of molecular variance (AMOVA) of the 188 Finger millet germplasm collection samples genotyped

Analysis of molecular variance revealed that there is a 48% variation within the genotyped finger millet germplasm collections samples and a 53% variation among the genotyped finger millet germplasm collections samples as shown in Figure 6. This is in the same range with the phenotypic data, which revealed a total variation of 42% among the finger millet genotype collections using principal component analysis.

Pairwise Nei’s standard genetic distance between the six populations of finger millet evaluated using 23 SNP markers.

Nei genetic distances revealed the closest relationship among the germplasm collection and the smaller the distance, the closer the relationship while the larger the distance the wider the relationship. The pairwise Nei’s standard genetic distance ranged from 0.059 to 0.388 among the six finger millet populations. The lowest genetic distance was found between Pop 4 vs Pop 6 (0.059) followed by Pop 4 vs Pop 3 (0.067), Pop 5 vs Pop 4 (0.085) and Pop 1 vs Pop vs Pop 3 (0.095). The highest genetic distance was found between Pop vs Pop 1 (0.349), Pop 2 vs Pop 3 (0.388) and Pop 2 vs Pop Pop1 (0.773) as shown in figure 3.

Table 3: Pairwise Nei’s standard genetic distance between the six populations of finger millet germplasm collections evaluated using 23 SNP markers.

	Pop 3	Pop 6	Pop 1	Pop 2	Pop 4	Pop 5
Pop 3	-					
Pop 6	0.108	-				
Pop 1	0.095	0.252	-			
Pop 2	0.388	0.111	0.773	-		
Pop 4	0.067	0.059	0.171	0.214	-	
Pop 5	0.225	0.153	0.349	0.223	0.085	-

NB: Pop means Population which are the six clusters based on geographical distribution

Geographical distribution of the 188 finger millet germplasm collections samples based on cluster analysis

Results revealed that out of the 188 germplasm collection samples genotyped, NPGRC contributed highly to the cluster groups with a total of 62 germplasm collections samples followed by Kandeu EPA with 38 germplasm collections samples and the least was Manjawira EPA with 6 germplasm collection samples as shown in table 4. Cluster 1 had a total of 22 germplasm collection samples with Kandeu EPA

contributing highly with 11 germplasm samples. Cluster 2 had a total of 10 germplasm collection samples, cluster 3, 4, 5 and 6 had a total of 61, 43, 26 and 26 germplasm collections samples respectively with Icrisat, NPGRC, Kandeu EPA contributing highly.

Table 4: Geographical distribution of the 188 finger millet germplasm collections based on cluster analysis

	Kandeu EPA	Manjawira EPA	Mombezi EPA	Chipala EPA	Eswazini EPA	NPGRC	Icrisat	Total
Cluster 1	11	1	3	3	1	2	1	22
Cluster 2	1	2	1	0	0	1	5	10
Cluster 3	9	3	6	9	11	23	0	61
Cluster 4	14	0	7	7	1	13	1	43
Cluster 5	1	0	0	2	7	14	2	26
Cluster 6	2	0	10	0	4	10	0	26
Total	38	6	27	21	24	62	9	188

Discussions

Qualitative trait variation

The germplasm collections under study revealed the presence of substantial variation based on agro morphological traits. This can be used for discrimination of unique traits by famers during seed production and selection by plant breeders. According to figure 1a, the most common plant color variation among the 40 finger millet germplasm collections was no pigmentation accounting for 77.5% while pigmentation accounted for 22.5%. Similar studies reported agro-morphological variations in traits such as plant pigmentation, growth habit, ear shape, and seed color in finger millet germplasm accessions. This was demonstrated in a study of 139 finger millet farmer accessions from Sri Lanka on plant pigmentation by Wijayasiri et al., (2018) who reported that 65.3% of the germplasm accessions were of the green type while the remaining germplasm accessions were of the pigmented type. A similar study by Gauchan et al., (2020) reported that 80% of the finger millet germplasm accessions had pigmented plants. In the study, 82.5 % of the 40 germplasm collections studied exhibited prostrate growth habits while 17.5 % exhibited erect growth habits as shown in figure 1b. A similar study by Kumar et.al,

(2019), reported variations in 16 qualitative traits that were visually evaluated on 92 finger millet germplasm accessions. In the study, 32.58% of the 92 observed germplasm accessions demonstrated decumbent growth habit while the remaining 50% and 17.39% of the germplasm accessions demonstrated erect and prostrate growth habits respectively (Kumar et.al, 2019). In a similar study on 150 germplasm accessions of finger millet, erect growth habit was found to be significantly higher (59.1%) than decumbent growth habit (27.3%) and prostrate growth habit (13.2%) (Azad & Manai, 2019).

Ear shape and size are important yield contributing traits that can also be used to identify sub-races. In this study, semi-compact ears accounted for 52.5 % of the 40 germplasm collections, with open type ears accounting for 37.5% and compact and fist-like type ears accounting for 5% as shown in figure 1c. Other researchers has been reporting variations in ear shape and seed color of finger millet germplasm accessions. Kumar et al., (2019) reported that germplasm collections with droopy type ears were the most common, accounting for 27.14 % of all entries, followed by semi compact (23.91 %), open (22.82 %), and fist-like type (26.13 %). Another study reported that 39% of the 300 accessions had open ears and 37% had semi-compact heads. (Gauchan et al., 2020). Farmers preferred compact ear type because of high grain yield, less shattering, blast tolerance, and less bird damage because they are well covered in their glumes. (Owere et al., 2014).

Seed colour is the most important trait in morphological identification, quality determination, and variety acceptance by smallholder farmers (Azad & Manai, 2019). This study that 50% of the germplasm collections had light brown colour, 45% had copper brown colour 2.5% had white colour and purple brown colour respectively as shown in figure 1d. In a similar study, Kumar et al., (2019) reported that there was no white seeds found in any of the germplasm accession, but light brown seeds were the most common with 55.43 %, followed by copper brown with 28.26 % and dark brown with 28.26 % whereas Upadhyaya et al., (2007) reported that out of 909 finger millet genotypes collected from Southern and Eastern Africa, light brown colour accounted for 57.2 % of the accessions, reddish brown had 22.3 % , dark brown had 10.2 % while brown and white seed colour exhibited for 8.4% and 1.9 % respectively. Gauchan et al., (2020) reported that 55% of 300 finger millet accessions had light brown seed color while 42% had purple brown seed color. Owere et al., (2014), reported that seed colour improved adoption rate of finger millet varieties. Results from baseline survey conducted in Malawi revealed that farmers preferred brown seed colour, for example, because of its good malting ability for brewing local beer. Dark brown colours were also less likely to be attacked by birds in the field because the seed was not visible to birds, as opposed to white seed colours, and darker grain colours had lower blast incidences (Owere et al., 2014). These traits can be used by breeder in the crop improvement program and increase adoption of the new varieties by farmers (Julong et al., 2017)

Principle Component Analysis (PCA) of the 14 agro-morphological traits.

PCA revealed a total variation of 42 % as shown in table 2. The four variables that weighted higher and contributed positively to variation in the first principal component than the other variables are peduncle length (cm), number of fingers per plant, flag leaf of blade length (cm), and yield (kg ha^{-1}). The three

variables that weighted higher and contributed positively to variation than the other variables in the second principal component are number of culm branches, leaf sheath length (cm), leaf sheath width (mm), and leaf blade length (cm).

In a similar study, the first two principle components revealed 57 % variability among 16 finger millet accessions as reported by Kandel et al., 2019,. Plant height (cm), number of productive tiller, number of days to flowering, flag leaf length (cm), peduncle length (cm), number of fingers per head, finger length (cm), and grain yield contributed the majority of variation. Finger length (cm) and width (mm) were found to have a significant influence on grain yield per plant in some studies conducted among finger millet genotypes from various regions of India (Dramadri et al., 2012). Furthermore, previous research reported that high variability in finger millet characteristics such as number of days to 50% flowering and maturity, plant height (cm), number of productive tillers, number of fingers per ear, and grain yield could be used in selecting diverse parents for hybridization breeding (Kinfé et al., 2017).

The PCA analysis showed how each trait involved in the development of the individual collection contributed to the total genetic variation observed among finger millet accessions. Thus, this shows that the finger millet germplasm collections are highly diversified for ears and maturity traits, which could be further utilized as a basis for selecting parent for crop improvement program. The recent findings suggested that indirect selection based on peduncle length (cm), finger width (cm), number of days to flowering, leaf sheath width (mm) and yield (kg ha^{-1}) would be useful in identifying superior germplasm collections for a finger millet crop improvement program (Owere et al., 2014).

Biplot of principal component analysis based on 14 qualitative traits.

The principal component of a biplot represents variables superimposed on a plot as vectors, where the relative length of the vectors represents the relative proportion of variability in each variable represented on the biplot (Uba et al., 2021). The biplot analysis revealed that yield (Kgha^{-1}), number of fingers per plant, leaf blade length (cm), leaf sheath length (mm), number of leaves per plant, plant height (cm) and peduncle length (cm) were important in differentiating the germplasm collections because they had relatively long vectors. In other words, they are the best discriminators of the morphological data under consideration. Number of days to flowering, flag leaf of blade length (cm), and flag leaf of blade width (cm), on the other hand, had the shortest vectors, indicating that they may be less important in evaluating finger millet germplasm collections for yield potential. Similar findings were also reported by Kandel et al., (2019) in germplasms of finger millet. Through the use of appropriate selection indices, knowledge of the interrelationships between grain yield and its associated traits will improve the efficiency of breeding programs (Anuradha et al., 2020). The magnitude of the vectors indicates the extent to which they contribute to each Principal Components. Vectors pointing in the same direction indicate positively correlated traits, vectors pointing in opposite directions indicate negatively correlated variables and vectors pointing at roughly right angles indicate low or no correlation. (Ban et al., 2021).

SNP characteristics

The SNP markers had an average Minor allele frequency (MAF) of 0.15, Neis genetic diversity (GD) of 0.2, observed heterozygosity (H_o) of 0.11, polymorphic information content (PIC) of 0.16 and an inbreeding coefficient (F_i) of 0.51. This is in agreement with a research by Brhane et al., 2022 who reported that the mean MAF was 0.19. The PIC of the SNPs varied from 0.09 to 0.38 which is in the same range with the current study (Brhane et al., 2022) while in a study by Gimode et al., (2016), the PIC ranged from 0.16 to 0.77 with an average of 0.42. PIC is a measure of the informativeness of molecular markers such that the higher a locus's PIC value, the more informative it is (Shete et al., 2000). When both alleles are equally frequent, a bi-allelic SNP locus can have a maximum PIC value of 0.38 (Brhane et al., 2022). The average PIC (0.16) obtained in the current study was lower than the average PIC (0.29) value estimate for finger millet. Brhane et al., (2022) also reported that the SNPs had an average observed heterozygosity 0.10 which is in agreement with the current study. Observed heterozygosity (0.11) was lower in the current study as compared to similar bi-allelic studies on Red clover (*Trifolium pretense*) which had an observed heterozygosity of 0.22. Red clover is strictly outcrossing enabling a higher level of heterozygosity (Osterman et al., 2021). Since finger millet is self-pollinating, there is a high likelihood of heterozygote deficiency at the greater part of the loci, though other evolutionary forces may have also played a role (Brhane et al., 2022).

Cluster analysis of the 188 germplasm collection samples genotyped

In the current study, genotypic data grouped the 188 germplasm collection samples into 6 clusters where the largest cluster has 61 germplasm collections while the smallest cluster has 10 germplasm collections. This is in agreement with previous research by Ramakrishnan et al., (2016) who reported that finger millet genotypes were grouped into 3 clusters. The cluster analysis results revealed that the subpopulations had allele admixture since some sub population had germplasm collections from more than one geographical area. Previous research reported that accessions in Subpopulations came from both southern Asia and eastern Africa (Kumar et al., 2016, Ban et al., 2021).

Analysis of molecular variance (AMOVA) of the 188 Finger millet germplasm collection samples genotyped

In the current study, genetic analysis revealed a 48% variation within and a 53% variation among the genotyped finger millet germplasm collections. This is in agreement with previous findings by Haftom et al., (2017), who reported 58.54% of variation within population and 41.45% among population. Similar findings by Brhane et al., (2022) reported that AMOVA for the seven finger millet revealed a total variation of 70% and 30% within and between populations respectively. Low genetic variation among germplasm collections revealed lower migration of finger millet and farmer selection of specific agronomic traits, which promotes local adaptation and genetic drift.

Pairwise Nei's standard genetic distance between the six populations of finger millet germplasm collections evaluated using 23 SNP markers.

Nei genetic distances revealed the closest relationship among the germplasm collection and the smaller the distance, the closer the relationship while the larger the distance the wider the relationship (Kumari & Pande, 2010). The pairwise Nei's standard genetic distance ranged from 0.059 to 0.388 among the six finger millet populations. The lowest genetic distance was found between Pop 4 vs Pop 6 (0.059) suggesting that these two populations are genetically similar and the highest genetic distance was found between Pop 2 vs Pop Pop1 (0.773) as shown in table 3. This is in agreement with a study conducted by Brhane et al., (2022) who reported that the pairwise Nei's standard genetic distance ranged from 0.01 to 0.48 among the seven finger millet populations. Similar findings were also reported for sorghum, another self-pollinating crop (Enyew et al., 2022). The information is important because the most diverse germplasm collections can be used for hybridization in plant breeding programs (Brhane et al., 2022).

Geographical distribution of the 188 finger millet germplasm collections based on cluster analysis

Genetic analysis in the current study revealed that there was a variation in number of germplasm collections per geographical area. Genebank contributed highly to the cluster groups with a total of 62 germplasm collections followed by Kandeu EPA and the least was Manjawira EPA as shown in table 4. This was reported in previous research where there was some degree of geographical clustering within the major clusters (Gimode et al., 2016, Kumar et al., 2016). Gimode et al., (2016), reported that some of the improved varieties deviated from the major cluster due to gene flow between *Subsp. africana* and *coracana*, as well as the recent history of finger millet breeding in eastern Africa, which was influenced by Indian accessions. Kumar et al., (2016) reported that the subpopulations had admixtures of alleles ranging from 10 to 55%.

Conclusions

Characterization of finger millet germplasm collections reveals the country's germplasm variability based on agro morphological traits. Three principal components explained 42% of the total phenotypic variation among the germplasm collections while AMOVA revealed that there is a 48% variation within the finger millet germplasm collections and a 53% variation among the finger millet genotype collections. Genotypic data grouped the 188 samples into 6 clusters. The pairwise Nei's standard genetic distance ranged from 0.059 to 0.388 among the six finger millet populations. A breeder's knowledge of existing genetic diversity and how to use it effectively is critical for efficient crop genetic resource management. Therefore, integrating conventional breeding with molecular breeding techniques can accelerate variety development and deployment, particularly for neglected and underutilized crops species like finger millet. This could significantly shorten breeding cycles and allow varieties to be released more quickly.

Declarations

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Competing interest

The author declare that there is no conflict of interest exist.

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Data availability

All relevant data are available within the manuscript and its supporting information files in the supplementary section.

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Figures

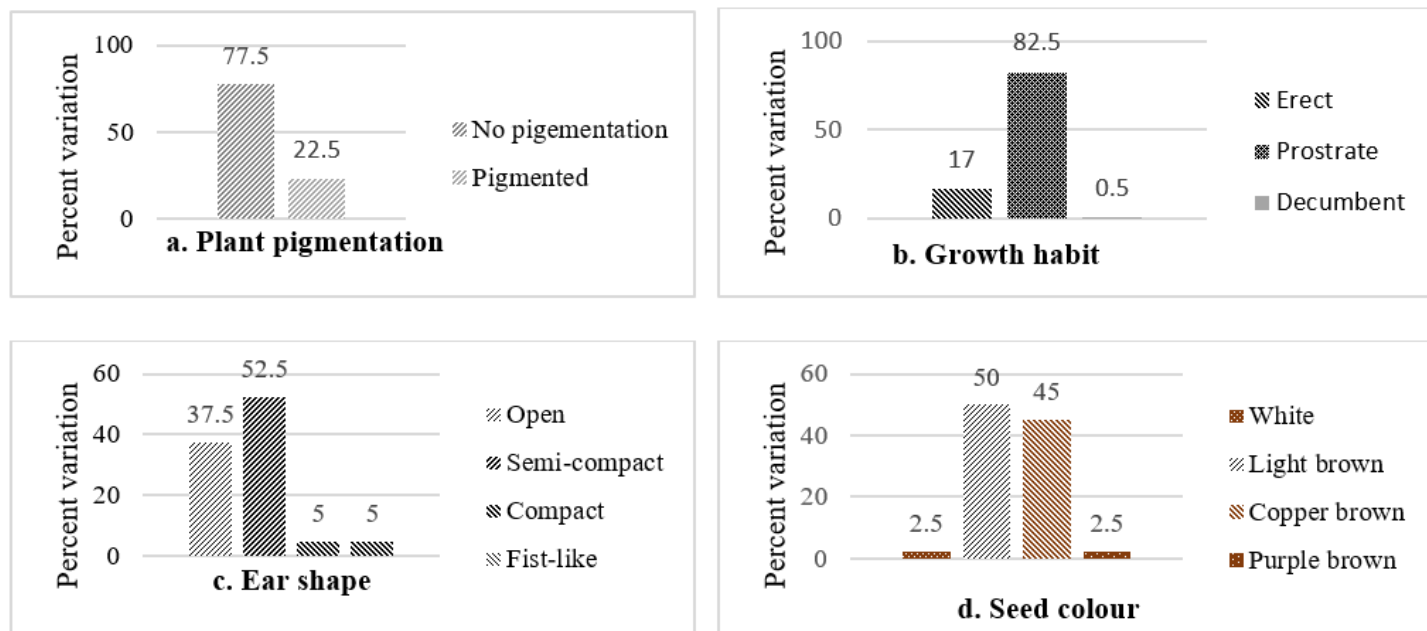


Figure 1

Qualitative trait variation (a) Plant pigmentation, (b) Growth habit, (c) Ear shape and (d) Seed colour for 40 finger millet germplasm collections evaluated at Bunda during 2020/2021 growing season.

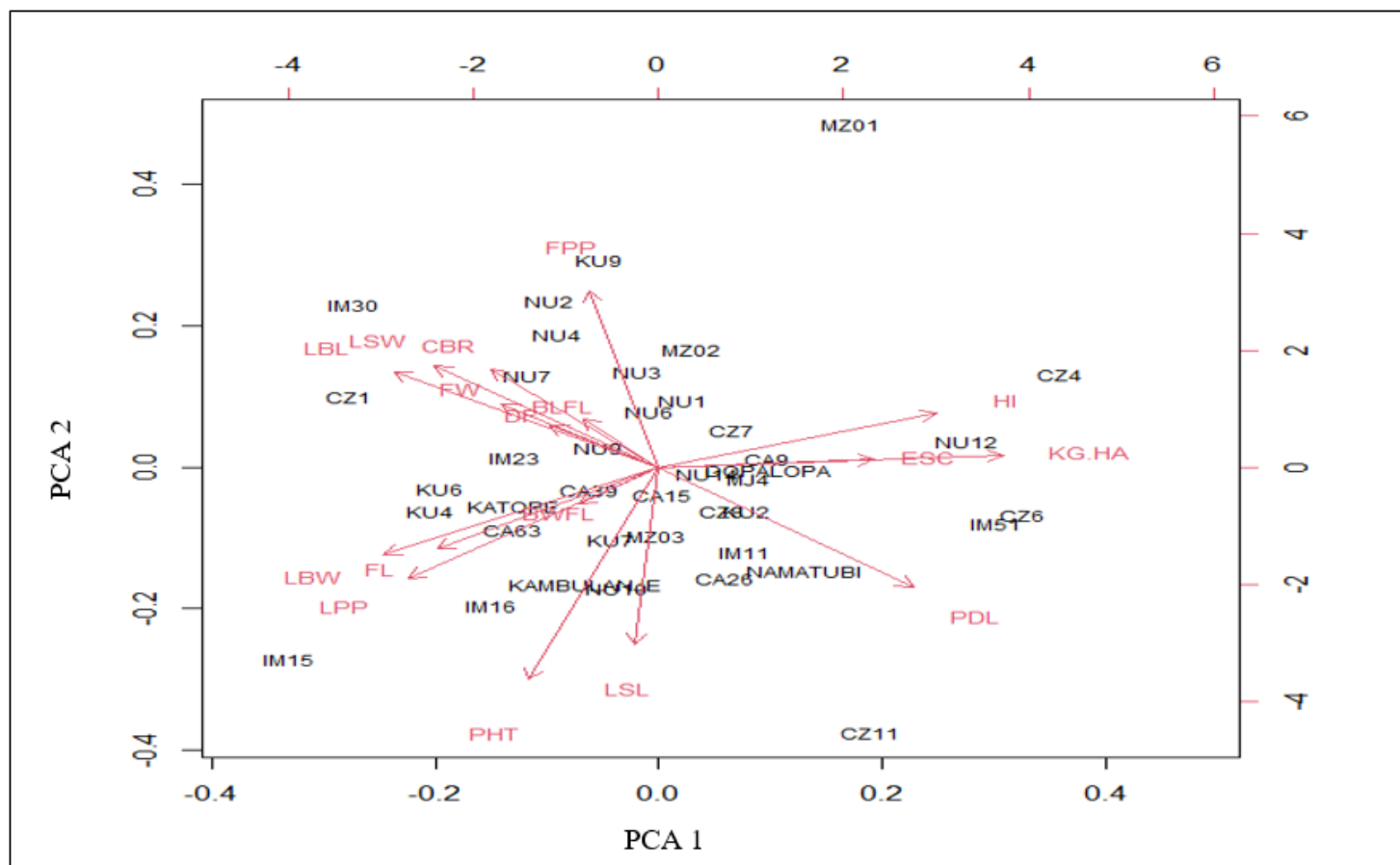


Figure 2

Biplot of principal component analysis based on 15 qualitative traits measured 40 finger millet farmer collections evaluated at Bunda during 2020/2021 growing season. FPP (Fingers per plant), LBL (Leaf blade length, LWB (Leaf blade width), LSW (Leaf sheath width), LSL (Leaf sheath length), CBR (Culm branching), FW (Finger width), FL (Finger length), DF (Days to flowering), BLFL (Blade length of flag leaf), BWFL (Blade width of flag leaf), LPP (Leaves per plant), PHT (Plant height), PDL (Peduncle length), HI (Harvest index), ESC (Emergence stand count) and KG.HA (Kilograms per hectare).

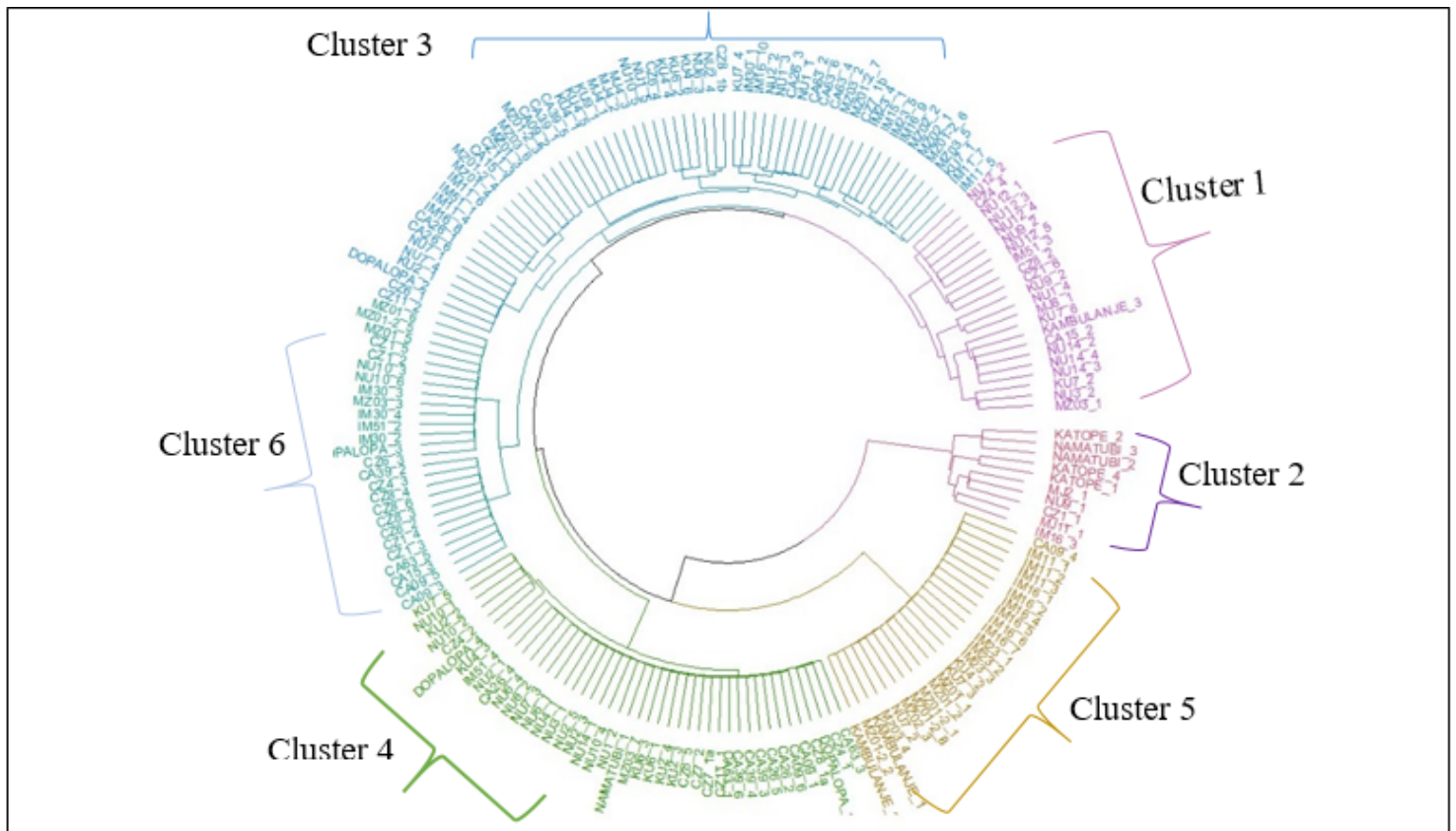


Figure 3

Fig 5: Dendrogram showing six cluster groups of the 188 germplasm collection samples genotyped

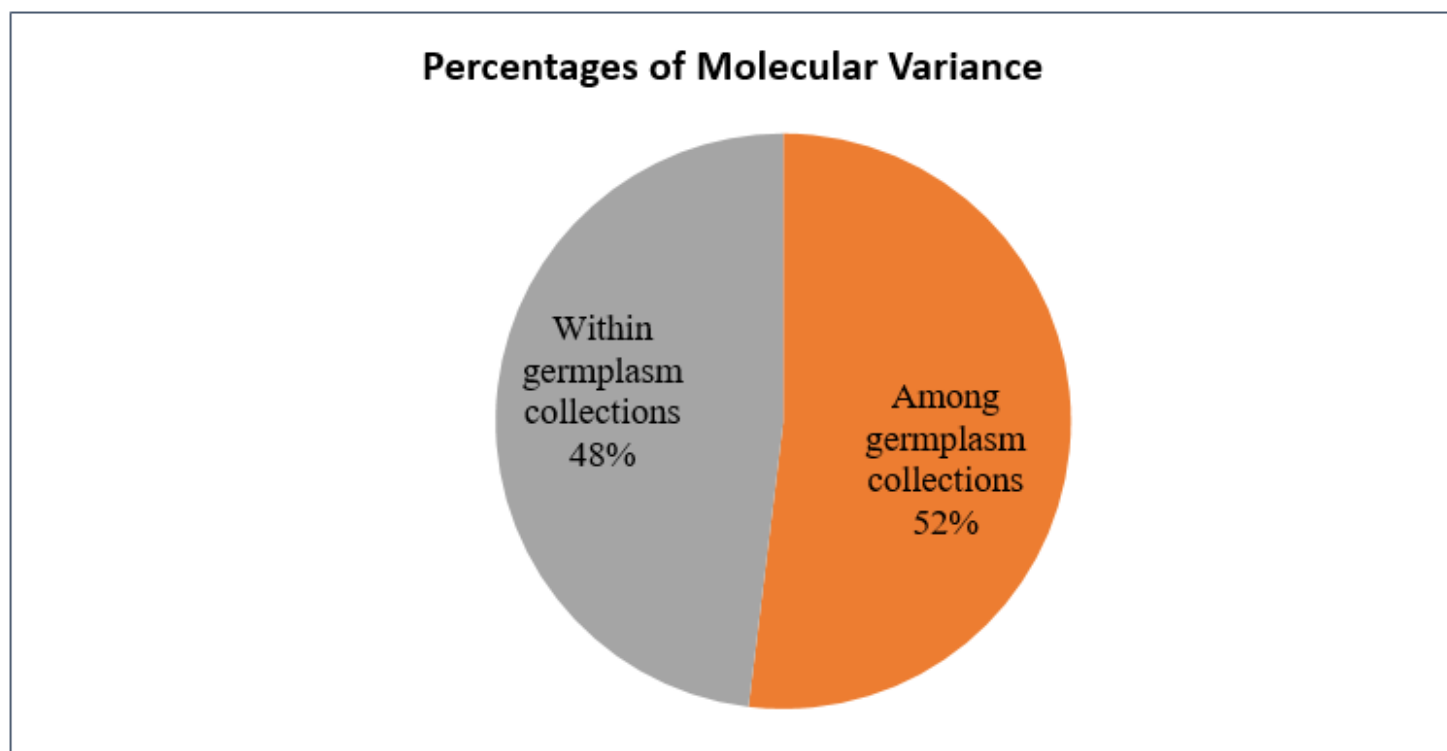


Figure 4

Fig 6: Percentages of Molecular Variance of the 188 Finger millet germplasm collection samples genotyped

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