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RESEARCH ARTICLE

Genome-wide association analysis revealed genetic markers linked to grain yield and yield related traits in finger millet grown in acidic soils

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Abstract

Aim Soil acidity has a major impact on the finger millet yield and productivity as tolerant cultivars that perform well in acidic soils are limited. This study aimed at evaluating major finger millet phenotypic traits under acidic soils followed by identifying associated markers.

Method A total of 288 finger millet genotypes were field evaluated for 8 major phenotypic traits including grain yield under acid soil conditions at two independent locations (Bako and Gute) in Ethiopia. In parallel, the same genotypes were subjected to genotyping-by-sequencing to generate single nucleotide polymorphism markers to be used in the association panel.

Results Phenotypic data analysis revealed significant phenotypic variation in all the targeted traits among the studied genotypes. Genotypes Ec-100093, Ec-215803, and Ec-203322 were relatively high-yielding, whereas genotypes Ec-229721 and Ec-242110 had the lowest grain yield across the two locations. The broad-sense heritability of the traits ranged from 0.04 for the number of effective tillers (NET) to 0.78 for days to emergence (DE). The marker-trait association analysis revealed 23 SNP markers significantly associated with one or more traits. Among the 23 significant markers, one marker associated with DE, seven with days to heading (DH), four with days to maturity (DM), one with plant height (PH), two with number of fingers, two with ear length (EL), three with the number of effective tillers (NET) and three with grain yield (GY).

Conclusions The identified novel markers associated with the targeted traits will potentially be useful for genomics-driven finger millet improvement in acidic soils.

Keywords Acidic soil · Finger millet · GBS · Linkage disequilibrium · Marker-trait associations

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Introduction

Finger millet (*Eleusine coracana* L. Gaertn.) is a small seeded cereal crop cultivated in most arid and semi-arid regions of Africa and Asia for food and animal feed (Goron and Raizada 2015). It is the third most important millet crop after pearl millet and foxtail millet in the world (Singh and Upadhyaya 2015). It is a hardy crop capable of providing reasonable grain yields under biotic and abiotic stresses where most other crops generally fail, and it is often considered a component of food security crop (Kinfe et al. 2017). Finger millet originated in East Africa, particularly Ethiopia and Uganda, and then extended to India about 3000 years ago (Hilu and De Wet 1976). In Ethiopia, finger millet is widely grown and used by small-scale farmers (Admassu et al. 2009; Kinfe et al. 2017).

Finger millet is a disomic tetraploid ($2n=4x=36$; AABB genome) crop and is considered to be originated from hybridization between the diploid species *E. indica* (L.) Gaertn ($2n=2x=18$; AA genome) and *E. floccifolia (Forssk.) Spreng* ($2n=2x=18$; BB genome) (Bisht and Mukai 2001). It exhibits excellent phenotypic diversity in grain color (dark brown, light brown, radish brown, and white), growth habit (erect, recumbent, and prostrate), panicle shape (open, semi-curved, and curved), and flowering time (Upadhyaya et al. 2007).

Globally, soil acidity ($pH \leq 5.5$) is one of the abiotic factors that limits crop yield and productivity (Von Uexküll and Mutert 1995). It has been estimated that 50% of the world's and over 40% of Ethiopia's potentially arable lands are acidic (Von Uexküll and Mutert 1995; Abdenna et al. 2007). In Ethiopia, soil acidity is expanding in coverage and severely limiting crop yield and productivity especially in the highly productive areas (Tesema 2008; Wassie and Shiferaw 2011; Trunhe and Yli-Halla 2016). For instance, in western Ethiopia the degree of soil acidity is becoming very critical and reached a level that affects crop productivity including finger millet (Abdenna et al. 2007; Trunhe and Yli-Halla 2016). Recently, (Seifu et al. 2022) also reported that 80% of the Nitisols soils found in the western high lands of Ethiopia are very strong to strongly acidic soils having pH of 4.5–5.5.

Generally, crop improvement involves various complex activities including utilization of existing genetic variation in desired traits and marker-trait associations (MTA) (Mnyenyembe and Gupta 1998). However, research on finger millet has been sparse, mostly focusing on genetic diversity using different morphological (Daba and Haile 2000; Tesfaye and Mengistu 2017), DNA markers (Lule et al. 2018; Brhane et al. 2021, 2022a), and RNA-seq analysis (Brhane et al. 2022b). One among many factors that have negatively affected advanced research, such as MTA analysis and genetic improvement of finger millet was a lack of reference genome assembled at a chromosome level. Recently, a chromosome-level assembly of the finger millet genome has been published, opening up opportunities for advanced research and genomics-driven breeding in this crop (Devos et al. 2023). Single nucleotide polymorphisms (SNPs) have been used to conduct genome-wide association (GWAS) research in various crops including finger millet due to their abundance, co-dominant nature, and high reproducibility (Sharma et al. 2018; Puranik et al. 2020). GWAS relies on next-generation sequencing-based genotyping-by-sequencing (GBS) that allows simultaneous SNP discovery and genotyping of target populations (Elshire et al. 2011). GBS provides a large number of SNP markers that can be used for MTA (Sharma et al. 2018; Kavuluko et al. 2021; Saleem et al. 2022).

Nowadays, GWAS is the most widely used approach for associating phenotypic traits with the underlying genetic background of various crops (Ward et al. 2019). In finger millet, few research has been published on MTA to identify genomic/scaffold regions governing nutritional traits, blast resistance genes, seed protein governing genes, and genes underlining grain iron (Fe) and zinc (Zn) (Sharma et al. 2018; Puranik et al. 2020; Tiwari et al. 2020; Sood et al. 2023; Chandra et al. 2024). Due to its statistical simplicity and speed of computation, multi-locus mixed model (MLMM) based Bayesian-information and linkage-disequilibrium Iteratively Nested Keyway (Blink) with sub-setting kinship estimation mostly have been employed in MTA analysis (Huang et al. 2019). In addition, Blink also eliminates the assumption that causal genes are evenly distributed across the genome, thereby increasing statistical power (Huang et al. 2019).

However, no research has been done to uncover the genomic regions and genes regulating yield and yield-related traits of finger millet grown under acidic

soil conditions. Hence, this study aimed at evaluating major finger millet phenotypic traits through replicated field trials at two locations with acidic soils, followed by GWAS utilizing the phenotypic data and GBS-based SNP marker data. This was done to identify favorable alleles, which could be incorporated into finger millet cultivars through breeding, thereby enabling breeders to improve the crop's grain yield in acidic soils.

Materials and methods

Plant materials

In this study, 288 accessions of finger millet were used; of which 274 accessions were obtained from the gene bank of the Ethiopian Institute of Biodiversity (EIB, Addis Ababa, Ethiopia) while the remaining 14 accessions were cultivars obtained from Bako Agricultural Research Center (BARC) Bako, Ethiopia (Supplementary Table 1). Among the gene bank accessions, 228 were landraces originally collected from various regions in Ethiopia, i.e., Amhara (130), Benishangul-Gumuz (2), Oromia (51), SNNPR (4), Tigray (32), and unknown locations (9). The remaining 46 gene bank accessions represent Zimbabwean finger millet genetic resources imported to Ethiopia for various purposes including research. For simplicity, the accessions will be referred to as "genotypes" herein.

Planting, leaf tissue sampling, genomic DNA extraction, and seed multiplication

The seeds of the 288 genotypes were sown in a greenhouse at the Swedish University of Agricultural Sciences (SLU, Alnarp, Sweden) to obtain an adequate number of progeny seeds for field trials and for genotyping. For each genotype, five seeds were planted in a 5 L plastic pot. Upon germination, extra seedlings were removed, and only one seedling was retained per genotype and used for genotyping and seed multiplication.

The leaf tissue of each genotype was collected separately three weeks after planting, DNA was extracted, and GBS-based genotyping was performed as described in Brhane et al. (2022a). To summarize, fresh leaf tissue samples were collected using the BioArk Leaf collection kit and then sent to LGC, Biosearch Technologies (Berlin. Germany) for genomic DNA extraction followed by GBS-based genotyping. High molecular weight genomic DNA was extracted using the Sbeadex plant kit (https://biosearch-cdn.azureedge.net/assetsv6/sbeadex-plant-data-sheet.pdf).

The seedlings were further grown to maturity under greenhouse conditions maintained at a light/dark cycle of 11/13 h day/night temperature of 18 °C and 16 °C, respectively and 65% relative humidity. Since finger millet is self-pollinating and one plant from each accession was sampled for genotyping, seeds harvested from individual plants were used in the field trials for acidity tolerance evaluation and phenotyping (described below).

Library construction, sequencing and read pre-processing

For the construction of a GBS library, *Pst*I (CTGCA*G, a 6-base cutter) and *Ape*KI (G*CWGC, a 4-base cutter) restriction enzymes were used following the recommendation of the experts at LGC Biosearch Technologies who optimized restriction enzymes for various crops. By combining *PstI-ApeKI*, a fragment size distribution suitable for sequencing on Illumina platforms was produced with a mean insert size of about 220 base pairs. The GBS reads were generated in 2 × 150 bp (paired-end) sequencing mode using NexSeq 500/550 v2 and NovaSeq SP FC NGS platforms. There were approximately 1.5 million read pairs per sample. After adapter-clipping, N-containing reads and reads that did not match the restriction enzyme site at the 5' end were discarded. To obtain an average Phred quality score of 20 or greater over a window of 10 bases, the 3'-ends of the remaining reads were trimmed using Trimmomatic v. 0.3 (Bolger et al. 2014), and reads with final lengths of less than 20 bases were discarded.

Read alignment, SNP discovery and genotype calling, and filtering

Since the recently released chromosome-level assembly of the finger millet genome (https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_032690845.1/) was not available by the time this study was carried out, the scaffold-level genome assembly (https://www.ncbi.nlm.nih.gov/assembly/GCA_00218

0455.1/) of the ML-365 cultivar was used as the reference genome (Hittalmani et al. 2017). An alignment of quality-trimmed reads against the reference scaffold-level assembled genome was performed using the BWA-MEM software package v. 0.7.12 (Li and Durbin 2009). Over 99% of the reads were mapped to the reference genome (which contained 525,627 scaffolds spanning 1.3 GB). For variant discovery and genotype calling in diploid format, Freebayes v. 1.2.0 (Garrison and Marth 2012) was used, and 101,889 SNPs were discovered across the 288 finger millet genotypes. GBS-specific criteria were then applied to filter the SNPs (minimum read count > 8, minimum allele frequency (MAF) of 0.05, and percentage of samples with assigned genotype > 66%) (Supplementary Table 3). Only bi-allelic markers used in the analysis. The data were further filtered, as described in Brhane et al. (2022a).

A total of 5226 bi-allelic SNPs having below 5% missing data and below 0.05 minor allele frequency (MAF) were used for the MTA analysis (Supplementary Table 3). To generate Manhattan plots, the 5226 SNPs were equally divided into nine scaffold groups (1–9) due to the lack of genome assembly at the chromosome level in finger millet at the time of genotyping. In silico analysis of the significantly associated markers was performed on the recently published chromosome-level assembly of finger millet (Devos et al. 2023).

Filed trials and phenotyping

The field trials were carried out during the 2021 main cropping season at two locations with acidic soils in Western Ethiopia where soil acidity is paramount: Bako Agricultural Research Center (BARC) and Gute Research Sub-Center (GRSC). BARC is located at 9°6′ N latitude and 37°09′ E longitude with an altitude of 1650 m.a.s.l, while GRSC is at 09°01.06′ N and 036°38.196′ E with an altitude of 1915 m.a.s.l. The dominant soil type in the locations is nitisol, which is characteristically reddish brown and loamy in texture with pH falling in the range of very strongly acidic to weak alkaline i.e., between 4.5 and 5.5 (Keba et al. 2022). They have similar patterns of rain during the rainy season from April to October (Kebede et al. 2019).

The experimental layout for the field trials was an alpha lattice design, comprising 17 blocks with 17 plots each, with two replications at both sites. Seeds of each genotype were sown by hand in two rows of 1 m length on each plot. The spacing between rows within a plot, between plots within a block, and between blocks within a replicate was 40 cm, 60 cm, and 80 cm, respectively. The distance between replicates within a site was 2 m. A seed rate of 15 kg ha^{-1} and fertilizer rate of 105 kg ha^{-1} DAP and 65 kg ha^{-1} urea were used at both sites. Plant management practices, such as hand-weeding were carried out according to adopted recommendations. The traits studied were days to emergence (DE), days to heading (DH), days to maturity (DM), plant height (PH), number of fingers (NF), ear length (EL), number of effective tillers (NET), and grain yield (GY). Phenotypic data of the traits were collected from five randomly chosen and tagged plants following the finger millet descriptor as described by Belete et al. (2020).

Phenotypic and genotypic data analysis

Prior to analysis, the 288 accessions were grouped into seven groups according to their geographic origin, which will be referred to as "populations" (Pop-1 to Pop-7) from here on for the sake of simplicity. Pop-1 to Pop-5 represent Ethiopian landrace accessions, and their descriptions are as follows: Pop-1 represents accessions collected from Agew-Awi, Gojam, Bahrdar, and Metekel (northwestern accessions); Pop-2 represents accession from western Tigray and Gonder (northern accessions); Pop-3 represents accessions collected from Wellega and Illuababora (western accessions); Pop-4 represents accessions from central, eastern, and southern Tigray and northern Wello (northeastern accessions); Pop-5 represents accessions with unknown sampling location; and Pop-6 represents the 14 improved cultivars whereas the 46 Zimbabwean landrace accessions form Pop-7.

Descriptive statistics, the best linear unbiased estimates (BLUEs), Best Linear Unbiased Predictions (BLUPs), broad sense heritability and correlation analysis of the phenotypic data within and across environments were estimated in R software (Supplementary Table 3).

The population structure and clustering analysis of the genotypes were performed using discriminant analysis of principal components (DAPC) approach using the adgenet package in R software (Jombart and Collins 2015). Bayesian Information Criterion

(*BIC*) for $K = 1 - 10$ (K = number of populations) was used to determine the optimal number of populations based on the minimum observed BIC value.

Stringently filtered SNP markers were used to conduct linkage disequilibrium (LD) analysis using Trait Analysis by Association, Evolution, and Linkage (TASSEL v. 5) with the default settings. Pairwise squared allele-frequency correlations (r^2) between SNP markers were generated, and the r^2 values were then plotted against the physical distance between the SNP loci to estimate the extent of LD between pairs of loci using R software.

Genome-wide association study (GWAS) was carried out to determine the association between phenotypic traits collected from field trials and stringently filtered GBS-derived SNP markers. This marker-trait analysis (MTA) was conducted using the Blink models in the Genome Association and Prediction Integrated Tools (GAPIT) package in R software (Lipka et al. 2012, Team 2013). For this purpose, we used 5226 bi-allelic SNPs with MAF above 0.05 and missing values below 5%. MTAs with $p \leq 0.001$ (i.e. $-\log10\ p \geq 3.00$) and false discovery rate (FDR) < 0.1 were considered significant. The percentage of phenotypic variance explained (PVE) by each marker was also estimated. The *p*-values from the model were used to generate Manhattan and quantile–quantile (Q-Q) plots using R software (Turner 2018). The sequences flanking the significantly associated SNP markers (149 kb upstream and downstream of the markers) were extracted and BLAST searched for sequence similarity against the Phytozome 13 *Eleusine coracana* v1.1 database (E. coracana v1.1: Phytozome (doe.gov)) using default parameters to identify putative candidate genes linked to the significant markers.

Results

General phenotypic performance of the genotypes

The analyses of finger millet phenotypic data collected from the two field experimental sites revealed a wide range of variation in the traits analyzed. The genotypes started emerging within 5 to 7 days, with most of them emerging (50%) within five days (Fig. 1A and Supplementary Table 2). In addition, the days to heading of the genotypes ranged between 82 to 110 days with an overall mean of 95 days

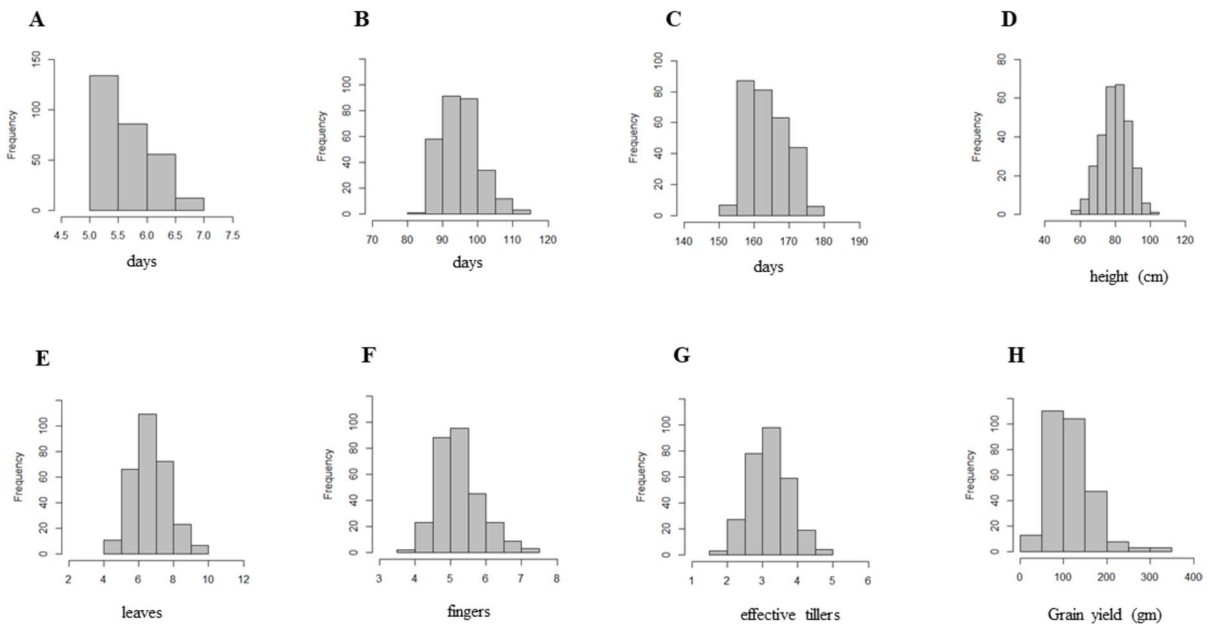

Fig. 1 Data distribution of the eight quantitative traits across 288 finger millet genotypes grown under acidic soils in Ethiopia. **A** DE; days to emergence, **B** DH; days to heading, **C** DM; days to maturity, **D** PH; plant height, **E** EL; ear length, **F** NF; number of fingers, **G** NET; number of effective tillers, and **H**) GY; grain yield

(Fig. 1B and Supplementary Table 2). Days to maturity (DM) varied from 152.5 and 176 with an overall mean of 163.3 days. The early maturing genotypes were Ec-211506, Ec-203272, and Ec-203327, reaching maturity in 152.5, 153.5, and 154.5 days, respectively. In contrast, the late maturing genotypes were Ec-243642, Ec-240506, Ec-215888, Ec-215850, Ec-215847, and Ec-208729, reaching maturity in 171.6 days (Fig. 1C and Supplementary Table 2).

The plant height (PH) of the genotypes varied from 59.2 cm to 101.2 cm, with an average of 80.01 cm. The top three tallest genotypes were Ec-2215952, Ec-216039, and Ec-203322 with 101.2, 98.4, and 97.4 cm, respectively, whereas the shortest genotypes were Ec-203344, Ec-203357, and Ec-237969 with 59.2, 59.9, and 61.1 cm, respectively (Fig. 1D and Supplementary Table 2). The number of leaves and the number of fingers were also counted from five individuals per genotype and he number of leaves per plant ranged from 4.5 to 9.5, while the number of fingers per plant between 3.7 and 7.5 (Fig. 1E and F, respectively). The number of effective tillers per plant across genotypes ranged from 1.7 to 4.7 (Fig. 1G and Supplementary Table 2). The highest grain yield (GY) was recorded from genotype Ec-100093 (322.7 g), followed by Ec-215803 (310.2 g) and Ec-203322 (301.2 g), while genotypes such as Ec-229721 and Ec-242110 showed lowest grain yields 31 g and 31.2 g, respectively (Fig. 1H and Supplementary Table 2). The overall mean grain yield of the genotypes was 115.6 g. The top three high-yielding genotypes were originally collected from Wollega (Ec-100093) and Gojam (Ec-215803), and Zimbabwe (Ec-203322). Among the cultivars Bako-09, Gute, and Wama performed better in the acidic environment than the other cultivars with grain yields of 150.6 g, 151.3 g, and 149.6 g, respectively. Performance of the least and top five genotypes in each traits were also given in Table 1.

Correlation analysis

Different correlations between the phenotypic traits was obtained and the highest significant positive correlation was observed between DH and DM (r = 0.78), followed by DE and DM (r = 0.66), and DE and DH

Table 1 List of five least and five top performance of the genotypes in each phenotypic traits evaluated under two independent acidic soil trials

Genotype	Performance	Trait	Genotype	Performance	Trait	Genotype	Performance	Trait	Genotype	Performance	Trait
215802	5.16	DE	216039	100.07	DH	215802	155.5	DM	238307	74.49	PH
203370	5.19	DE	215902	100.10	DH	203371	156.4	DM	203344	74.75	PH
203371	5.19	DE	216035	100.11	DH	203362	156.9	DM	219832	74.76	PH
335141	5.19	DE	230562	100.26	DH	215846	157.8	DM	215836	74.80	PH
203355	5.20	DE	216038	100.48	DH	215847	157.8	DM	203370	74.92	PH
245088	6.56	DE	215957	99.456	DH	215857	171.2	DM	203339	85.05	PH
216035	6.60	DE	215859	99.532	DH	215859	171.2	DM	215905	85.09	PH
242110	6.60	DE	215906	99.732	DH	208730	171.3	DM	216051	85.71	PH
215870	6.61	DE	215955	99.953	DH	215894	171.4	DM	215957	85.84	PH
243623	6.73	DE	215870	99.991	DH	242110	171.6	DM	100093	86.94	PH
203371	7.47	EL	215896	5.16	NF	208441	3.18	NET	215996	100.04	GY
203370	7.51	EL	215844	5.18	NF	Axum	3.18	NET	203377	100.54	GY
Tadesse	7.70	EL	203357	5.19	NF	203336	3.19	NET	238345	100.56	GY
203358	7.71	EL	242133	5.19	NF	203338	3.19	NET	242612	100.67	GY
203374	7.73	EL	203362	5.20	NF	203340	3.19	NET	242613	100.68	GY
242613	9.47	EL	228903	5.44	NF	Gudatu	3.25	NET	215832	98.772	GY
242135	9.49	EL	215911	5.45	NF	203335	3.26	NET	208730	98.925	GY
207460	9.52	EL	243644	5.47	NF	203370	3.26	NET	208941	99.439	GY
208443	9.52	EL	245087	5.49	NF	215919	3.26	NET	242689	99.486	GY
208440	9.68	EL	100093	5.51	NF	215933	3.26	NET	203335	99.508	GY

(r = 0.54) (Fig. 2). Plant height (PH) showed intermediate positive correlations for all traits except for NET and EL, which were not correlated with PH (Fig. 2). Moreover, GY was significantly positively correlated with PH (r = 0.23), EL (r = 0.32) and NET (r = 0.15), while no correlation was observed with the other phenotypic traits (Fig. 2).

Variance components and heritability

The combined variance components of the eight quantitative traits collected across 288 finger millet genotypes, grown at two sites with acidic soils, revealed significant variation among genotypes in all traits. Among the variance components, the genotypic variance was higher in DE, NET and GY traits compared to the environmental variance. However, it was lower in other traits, such as DH, DM, PH, and NF (Table 2). Comparatively, the broad sense-heritability (H^2) among the traits was higher in DE (0.86), followed by GY (0.68), DM (0.67), DH (0.49), EL (0.35), PH (0.33), while lower in NF (0.09) and NET (0.04) (Table 2).

Population structure and linkage disequilibrium

The discriminant analysis of principal components (DAPC) of the 288 finger millet genotypes based on

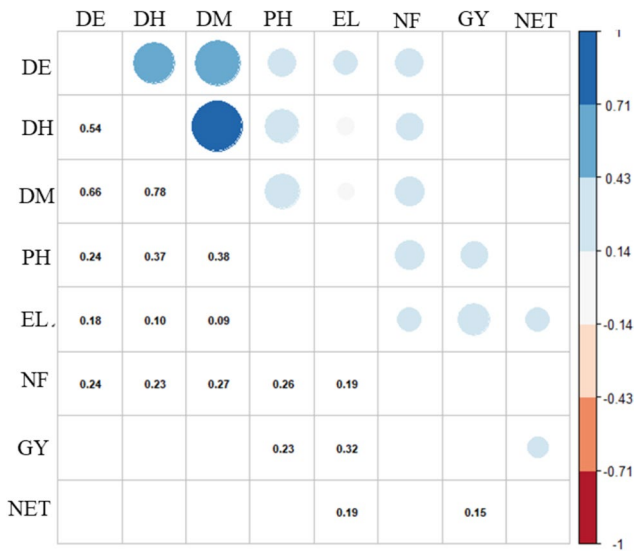

Fig. 2 Phenotypic trait correlation analysis of the 288 finger millet genotypes grown under acidic soil condition. Abbreviations: DE; days to emergence, DH; days to heading, DM; days to maturity, PH; plant height, EL; ear lengths, NF; number of fingers, NET; number of effective tillers, and GY; grain yield

Table 2 Estimation of the different variance parameters for eight major quantitative traits of 288 finger millet genotypes evaluated under two independent acidic soil trials

	Mean Squared Error								
Source of variation	DF	DE	DH	DM	PH	NF	EL	NET	GY
Environments (ENV)	1	0.01	31764*	26922*	5206*	253.00*	34.48*	0.28	8511*
Genotype (GEN)	284	1.107*	123**	104**	264**	1.52**	9.25**	1.27**	9122**
REP (Location)	2	18.75	405	89	1239	39.52	178.6	106.6	8899
GEN × ENV interaction	284	1.13	151*	72*	525*	5.95*	30.97*	4.62**	5934*
Residuals	811	0.30	52	31	144	1.21	1.21	0.94	2312
Heritability		0.86	0.49	0.67	0.33	0.09	0.35	0.04	0.68
Mean		5.8	95.3	163.2	80.01	5.31	5.31	3.22	115.7

**,* Significant difference at $P < 0.01$ and $P < 0.05$, respectively

DF Degrees of freedom, *DE* days to emergence, *DH* days to heading, *DM* days to maturity, *PH* plant height, *EL* ear length, *NF* number of fingers, *NET* number of effective tillers, *GY* grain yield

filtered SNP markers revealed that the genotypes were grouped into six clusters each representing different populations (Fig. 3A). Using the retained six clusters and 200 PCs, a scatter plot was generated to visualize the placement of each genotype in the scatter plot. Clusters I comprised of 17 genotypes (16 from Pop-3 and 1 from Pop-7), Clusters II comprised of 52 genotypes (45 from Pop-1 and 7 from Pop-2), and Clusters V comprised of 50 genotypes (3 from Pop-1, 1 from Pop-3, 2 from Pop-6, and 44 from Pop-7), respectively, and were dominated by genotypes from a single population. On the other hand, clusters III, IV, and VI comprised 33 genotypes (22 from Pop-1, 10 from Pop-3, and 1 from Pop-6), 48 genotypes (22, 17, 2, 6, and 1 from Pops-1, 2, 3, 5, and 7, respectively), and 88 genotypes (13, 7, 26, 28, 3, and 11 from Pops-1, 2, 3, 4, 5, and 6, respectively), and were more heterogeneous than the other clusters. The analysis revealed that genotypes from Zimbabwe were clustered separately, whereas weak clustering was observed for genotypes from Ethiopia (Fig. 3B).

The 5226 filtered SNP markers located across the finger millet genome scaffolds formed 154,626 SNP pairs. About 53,543 (34.6%) of the SNP pairs had $r^2 \geq 40\%$ while 19.2% were in LD ($r^2 = 0.1$, $P < 0.05$). The LD begins to decay at $r^2 = 0.46$ and drops to its half-decay at $r^2 = 0.1$ at a distance of 149 kb between marker pairs (Fig. 4).

Marker-trait association analysis

Marker-trait association analysis was conducted using the BLINK model, leading to the identification of 23 significantly associated single nucleotide polymorphism (SNP) markers across the eight traits of finger millet. Among the 23 SNP markers significantly linked to phenotypic traits, one SNP was associated with days to emergence (DE), seven with days to heading (DH), four with days to maturity (DM), one with plant height (PH), 11 with the number of fingers (NF), two with ear length (EL), three with the number of effective tillers (NET), and three with grain yield (GY).

The only one SNP marker associated with DE was LXGH01314605.1 which explained 0.52% of the total phenotypic variance. The minor allele frequency (MAF) of the marker was 0.50 and its LOD value was 3.08 (Table 3). Seven markers were associated with DH which explained phenotypic variance ranging from 0.56% to 6.50% and its MAF ranged from0.05

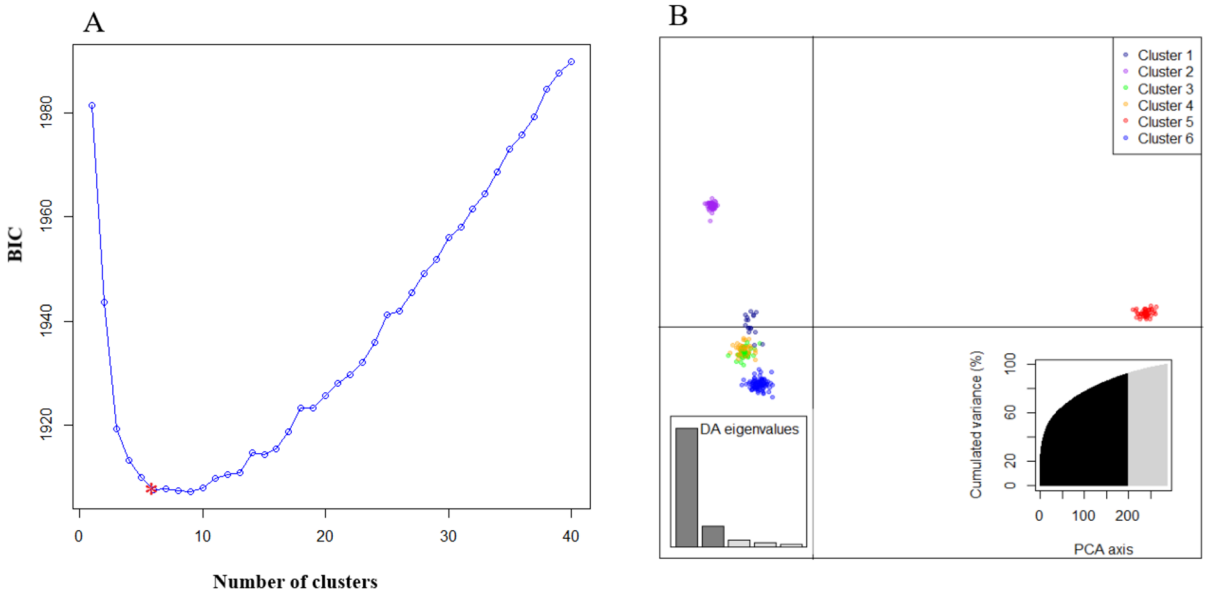

Fig. 3 Graphs depicting a discriminant analysis of principal components (DAPC) for 288 finger millet genotypes. **A** Optimum number of clusters indicating K = 6 and (**B**) Scatterplot of the genotypes using the first two principal components distinguishing six genetic clusters. The clusters were distinguished by colors. Discriminant analysis bar plots (bottom left) and principal components (bottom right) were also shown

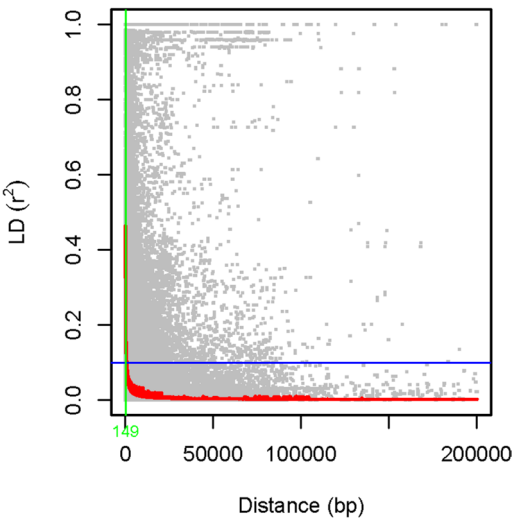

Fig. 4 A scatter plot of genome-wide linkage disequilibrium (LD) decay as determined by the r^2 values of the marker pairs. The smoothing spline regression model fitted to LD decay is shown with a red curve line. The horizontal blue line shows the genome's half-decay r^2 value ($r^2 = 0.1$), while the vertical green line shows the genetic distance between markers (149 Kb) at the intersection of the half-decay line and the LD decay curve

to 0.44. Four markers associated with DM explained phenotypic variance between 0.68% and 6.80% (Table 3). The significant marker markers associated with DE, DH, and DM, identified through the BLINK model, were visualized in Manhattan plots alongside their respective QQ plots in Fig. 5A, B, and C, respectively.

The MTA panel also identified one SNP marker significantly associated with PH ($P < 0.05$), with a minor allele frequency (MAF) of 0.06 (Table 3). This marker explained 0.89% of the phenotypic variance and its Manhattan plot and QQ plot provided in Fig. 5D. In addition, two SNP markers were linked with NF, explaining phenotypic variance between 0.62% and 0.80% (Table 3). The MAF of the significantly associated markers ranged from 0.05 to 0.48 (Table 3). These associations were depicted in a Manhattan plot with a corresponding QQ plot (Fig. 5E). Two SNP markers were also linked to EL, with Manhattan plots and QQ plots provided in Fig. 5F. Three SNP markers significantly associated with NET explained phenotypic variance between 0.54% and 0.80%, with MAF ranging from 0.05 to 0.27 (Table 3). These associations were illustrated in a Manhattan plot and QQ plot (Fig. 5G). Finally, three SNP markers were found to be significantly associated with GY, explaining phenotypic variance ranging from 1.38% to 30.8% (Table 3). The Manhattan plot and QQ plot for GY-related SNP markers are presented in Fig. 5H.

Alleles of the LXGH01314605.1 marker linked with days to emergence were AA and GG. Allele AA composed of 265 genotypes and allele GG composed of 23 genotypes. There was no significant difference among the genotypes explained by this marker for this trait (Fig. 6A). Among the alleles (AA, AG and GG) of LXGH01485647.1 marker which were associated with DH, genotypes with TT alleles exhibiting earlier heading compared to the genotype with AA alleles which started heading late (Fig. 6B). Among the markers that were associated with MD, LXGH01337172.1 explained the highest phenotypic variance. The alleles of this marker were AA, AG, and GG. The genotypes with GG allele showed late maturing compared to the genotypes with AA allele (Fig. 6C). LXGH01346160.1 marker was associated with PH and explained the highest phenotypic variance (0.94%). The allele of this locus were AA, AG, and GG. Genotypes with GG allele showed long in their height compared to the AA genotype that exhibit short (Fig. 6D). Allele combination of NF and EL provided in Fig. 6E and F. LXGH01052700.1 marker explained the highest phenotypic variation with the grain yield (30.8%). The alternative alleles of this marker were AA, AG, and GG, with AA genotypes exhibiting significantly high yields compared to genotypes with AG allele (Fig. 6H).

LXGH01314605.1 SNP markers was found shared between DE and NF and another LXGH01452763.1 marker was also linked between DH and GY. Table 3 provides details for 23 significant markers with PVE values greater than 0.5 including scaffold ID, SNP alleles, SNP position within a scaffold, *P*-value, and MAF for each trait. Among the significantly associated markers, those strongly associated with their corresponding traits and explained a relatively high proportion of phenotypic variance were annotated based on finger millet genomic resources in the public databases.

Table 3 List of 23 SNP markers significantly associated ($P < 0.05$) with finger millet traits targeted in this study that explained more than 0.5% of the total variance in their corresponding traits

Trait	Scaffold ID	alleles	Position	*P*-value	LOD	Effect	MAF	PVE (%)
DE	LXGH01314605.1	T/C	5542	0.004	3.18	-0.09	0.498	0.52
DH	LXGH01208436.1	T/A	29293	6.2E-05	4.20	-1.65	0.380	0.677
	LXGH01452763.1	A/T	113304	0.00029	3.53	-0.58	0.053	0.807
	LXGH01485647.1	A/T	1718	0.00050	3.30	-1.54	0.362	9.96
	LXGH01183671.1	T/C	43941	0.00054	3.26	-0.79	0.159	0.56
	LXGH01520281.1	A/G	3954	0.00069	3.16	2.19	0.156	6.50
	LXGH01099718.1	T/C	3758	0.00083	3.08	1.20	0.154	2.71
	LXGH01074376.1	G/T	440	0.00095	3.02	0.60	0.442	1.49
DM	LXGH01139662.1	G/T	19176	0.003	2.52	1.34	0.179	0.685
	LXGH01369512.1	A/G	5542	0.002	2.70	1.20	0.080	0.779
	LXGH01384244.1	C/G	9994	0.001	3.00	0.87	0.061	0.846
	LXGH01337172.1	A/G	20956	0.001	3.00	0.80	0.138	6.80
GY	LXGH01452763.1	T/G	49927	0.000	3.00	-99.8	0.053	1.387
	LXGH01052700.1	A/G	28450	0.011	3.00	-67.19	0.095	30.84
	LXGH01346143.1	C/G	32418	0.000	3.00	7.19	0.06	1.40
EL	LXGH01311505.1	A/G	2125	0.003	2.52	-0.19	0.054	0.838
	LXGH01501788.1	T/C	2354	0.007	2.15	-0.17	0.214	0.921
NET	LXGH01386342.1	T/C	28492	0.002	2.70	-0.005	0.068	0.546
	LXGH01038485.1	A/G	14232	0.002	2.70	-0.003	0.068	0.600
	LXGH01368953.1	T/C	39929	0.001	3.00	-0.012	0.229	1.475
NF	LXGH01314605.1	T/C	5542	0.011	2.00	0.018	0.146	0.624
	LXGH01281367.1	T/C	23526	0.007	2.15	0.023	0.149	0.888
PH	LXGH01346160.1	A/G	2819	0.008	2.09	1.02	0.06	0.888

MAF Minor allele frequency, *PVE* phenotypic variance explained, *DE* days to emergence, *DH* days to heading, *DM* days to maturity, *PH* plant height, *EL* ear lengths, *NF* number of fingers, *NET* number of effective tillers, *GY* grain yield

Discussion

In the present study, a wide range of grain yield variation (31–322.7 g) with an average yield of 115.6 g/m2 was obtained among finger millet genotypes grown in two independent fields with acidic soils. The observed variation in grain yield is in contrast with previously reported grain yield of finger millet grown at Adet and Finoteselam sites in Ethiopia, which yielded 239.7 g/m^2 and 238.6 g/m^2, respectively (Marefia et al. 2022). Likely, the difference in grain yields obtained in our study compared to the above report may be due to the acidic soils in Gute and Bako, while the soils at Adet and Finoteselam are not acidic and are therefore expected to produce higher grain yields. Interestingly, the top five high-yielding finger millet genotypes were originally collected from Wollega and Gojam (western Ethiopia), whereas most of the low-yielding genotypes were originally collected from Tigray and Gonder (northern Ethiopia). The genotypes originally collected from areas with acidic soils were high yielding, while those from areas with non-acidic soils were low yielding, and, hence, can be regarded as Al-tolerant and Al-susceptible, respectively. This could be mainly due to enhanced acidic soil of genotypes acquired through long-term exposure to acidic soils. Tolerance mechanisms could be by exclusion of Al from the root, sequestration of Al to different plant parts once it enters the plant cell, or both (Barcelo and Poschenrieder 2002; Kochian et al. 2005). Future research based on well-designed experiments will shed light on the tolerance mechanisms of the genotypes investigated in this current study. The consistently high-yielding genotypes across the two environments Ec-215829 (243.5 g), Ec-203322 (228.0 g), and Ec-100093 (213.5 g) should be considered in the finger millet breeding program for developing improved cultivars, particularly targeting acidic soils.

Heritability measures the transmission of characters from parents to their offspring (Falconer 1981). In this study, days to emergence, days to heading, days to maturity, and number of grains per spikelet showed higher broad sense heritability (H^2) compared

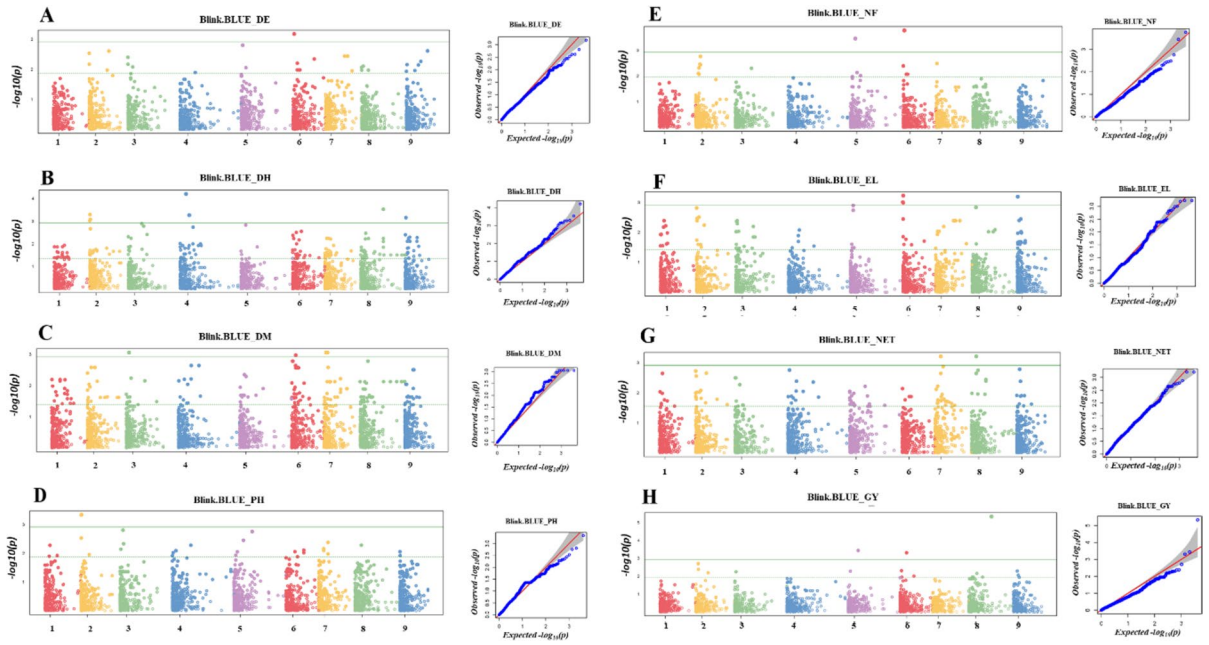

Fig. 5 Manhattan's and quantile–quantile plots produced from genome-wide association study (GWAS) in **A**) days to emergence (DE), **B** days to heading (DH), **C** days to maturity (DM), **D** plant height (PH), **E** number of fingers (NF), **F** ear length (EL)**, G** number of effective tilers (NET), and **H**) grain yield (GY) traits

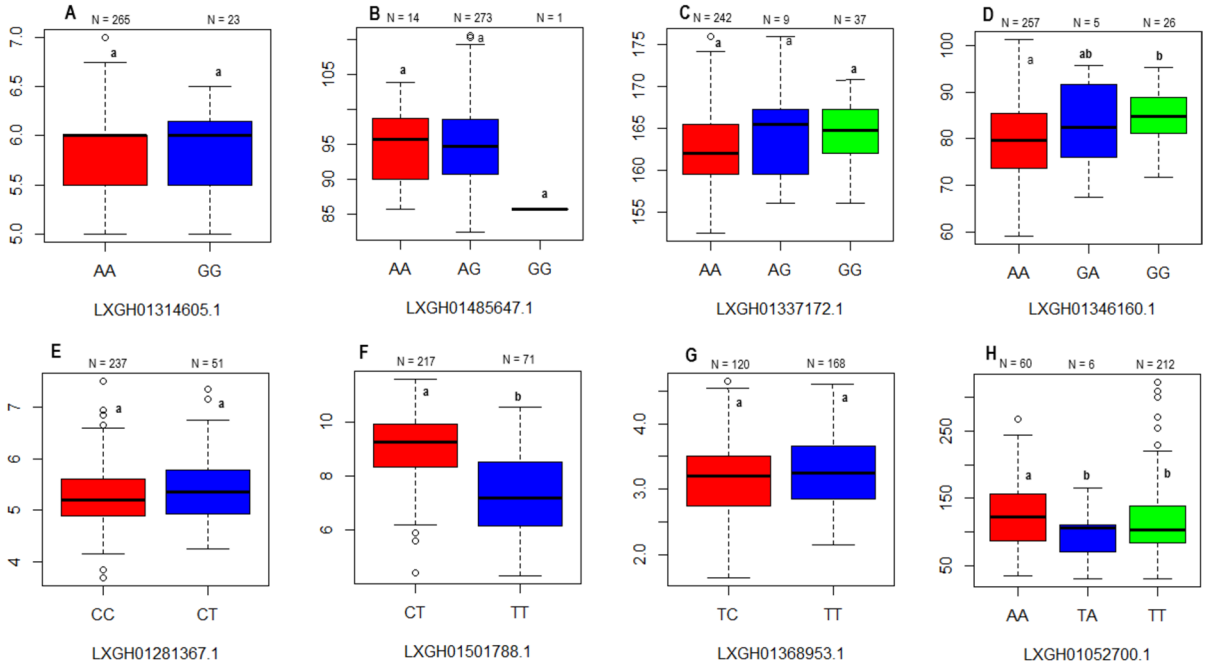

Fig. 6 Boxplot showing the allelic effect of significant marker-trait association with **A**) days to emergence (DE), **B** days to heading (DH), **C** days to maturity (DM), **D** plant height (PH), **E** number of fingers (NF), **F** ear length (EL), **G** number of effective tilers (NET), and **H**) grain yield (GY) traits

to plant height, ear length, and number of fingers. A similar heritability pattern among diverse finger millet germplasm was previously reported (Marefia et al. 2022). The moderate and high heritability observed in most of the targeted traits indicates that genotypes have higher effects than environments on their phenotypic variation, unlike traits with low heritability where environmental effects are high.

The correlations between most traits obtained in this study were in line with previous reports where significant positive correlations of DH with EL, DM, and PH were reported (Bharathi 2011; Sharma et al. 2018). Likewise, the positive correlation between PH and GY obtained in this study agreed with previous findings (Sivagurunathan 2004; Sharma et al. 2018). These investigations showed that finger millet genotypes that grow taller in acidic soils produce higher grain yields. Interestingly, this study revealed genotypes with desirable characteristics in major target traits including grain yield, plant height as well as tolerance to soil acidity. Hence, crossbreeding of genotypes with these desirable characteristics (high grain yield, tall height, and Al-tolerant) will increase the likelihood of recombination of alleles with positive effects on the phenotypes, and eventually developing superior cultivars.

The DAPC and population structure analysis showed that the genotypes originated from six genetic populations (K = 6), which is in line with previous findings in finger millet (Brhane et al. 2022a). The detailed analysis of genetic diversity and population structure of the genotypes used in this study, including their potential significance for finger millet improvement, was previously published (Brhane et al. 2022a). They represent genotypes from diverse agro-ecozones in Ethiopia and Zimbabwe, as well as cultivars released in Ethiopia. Overall, the genotypic and phenotypic variation, and population structure determined in this set of genotypes make it suitable for GWAS to identify genomic regions and candidate genes potentially associated with acidic soils.

Determination of LD between markers is an important step in GWAS, as it facilitates the selection of a set of markers suitable for efficient marker-trait association analysis. The LD between markers varies within a genome as well as between genomes of different crops, as it is affected by various factors, such as reproductive mechanism, rate of recombination, selection pressure, genetic drift, physical linkage, and population structure (Puranik et al. 2020). Since the LD analysis in the present study was based on SNP markers across a large number of scaffolds, it was difficult to estimate the markers' rate of LD decay accurately, as was also indicated in previous research (Sharma et al. 2018). In this study, about 19.2% of the SNP marker pairs showed significant LD ($r^2 > 0.1$; $P < 0.05$). A similar finding was reported by Sharma et al. (2018), who reported that 17.9% of finger millet SNP marker pairs were in LD (r2 > ; p 0.05). Another study on finger millet by Puranik et al. (2020) indicated that about 16.8% of finger millet SNP pairs were in LD ($r^2 \geq 0.2$; $p < 0.05$), and the maximum LD was dropped to its half (LD half-decay) at a distance of 28 kbp. Comparatively, genome-wide LD decay in foxtail millet ranged from 100 to 177 kb (Jaiswal et al. 2019).

In this study, 5226 GBS-derived SNP markers and eight phenotypic traits were used to identify significant marker-trait associations (MTA). The analysis revealed 23 novel SNP markers associated with one or more target phenotypic traits at highly significant levels. Sharma et al. (2018) reported 109 SNP markers associated with 14 finger millet agro-morphological traits including grain yield using SLST = 20, MLMM = 36, and MTMM = 53 models. Another GWAS study on finger millet identified 418 SNP markers associated with nutrition-related traits using GLM and mixed MLM models (Puranik et al. 2020). The relatively low number of MTAs identified in this study could be due to factors, such as the number of phenotypic traits studied and the stringency of the GWAS model used. Overall, this study showed that the GBS-derived SNP marker set used is suitable to identify potential markers suitable for marker-aided selection of genotypes for breeding finger millet for cultivation on acidic soils.

Among SNP loci associated with DH, an SNP marker located at position 1528 in scaffold LXGH01485647.1 explained the highest phenotypic variance in this trait. The alignment of this scaffold to the recently released finger millet chromosome-level assembly Devos et al. (2023) showed that this SNP is located on chromosome 5A within the ELECO. r07.5AG0380680 gene, which codes for Carboxyspermidine synthase /Carboxyspermidine dehydrogenase in finger millet. Carboxyspermidine synthase is an enzyme used for biofilm formation and cell viability in microbes through cationic polyamine

spermidine (Ko et al. 2022). Polyamine spermidine promote plant growth in part by the induction of systemic resistance (ISR) to biotic and abiotic stresses (Melnyk et al. 2019). The genotypes at this locus are AA, AT, and TT, which showed significant phenotypic variation in days to heading, and AA genotypes started heading later than TT and AT genotypes.

Among SNP loci significantly associated with GY, LXGH01052700.1_28450 was the most significant, as it explained 30.8% of grain yield variation. The locus is located within the ELECO.r07.1AG0026860 gene on chromosome 1A as per the recently published finger millet genome by (Devos et al. 2023). ELECO.r07.1AG0026860 is an orthologue of the Pgl_GLEAN_10002072 gene in pearl millet. Pgl_GLEAN_10002072 showed significant association with mass features mostly engaged in hydrolase activity, especially acting on ester bonds to promote carotenoid accumulation in pearl millet (Yadav et al. 2021). This suggests the gene plays a key role in pathways that promote the accumulation of antioxidant-related flavonoid compounds. The internal detoxification work in a variety of ways, such as hormone regulation, antioxidant defense system, and vacuole compartmentalization. As an important condition to maintain plant growth and development, the antioxidant defense system (enzyme and non-enzyme system) produces stress response to eliminate the excessive accumulation of reactive oxygen species in the plants (Sharma and Dubey 2007). This can ultimately improve grain yield.

The LXGH01346160.1_21363 locus showed a significant association with PH although it explained only 0.94% of the variation in plant height, which is the highest compared to that of other loci associated with this trait. This locus is located within the ELECO.r07.7AG0569730 gene on chromosome 7A. The ELECO.r07.7AG0569730 gene codes for late embryogenesis abundant protein (LEA_2) in finger millet. Late embryogenesis abundant protein, which is also characterized in various other crops, such as *O. sativa* (OsLEAs), *Z. mays* (ZmLEAs), a nd *S. bicolor* (SbLEAs) was shown to play an important role in plant stress adaptation (Wang et al. 2007; Li and Cao 2016; Nagaraju et al. 2019). When under abiotic stresses such as drought, cold, and salinity, these genes are expressed and play a protective role (Ingram and Bartels 1996). This suggests that the late embryogenesis abundant gene may be induced during Al-stress in finger millet to protect and promote plant growth.

Conclusions

The present study revealed high phenotypic and genetic variations among finger millet genotypes. A total of 23 markers associated with finger millet traits were obtained and could potentially be used for screening finger millet genetic resources for tolerance to acidic soils. However, these markers should be validated with further studies, particularly those that explain substantial phenotypic variations. Genotypes with high yield potential identified in this study can be used as parents in breeding programs aimed at pyramiding desirable alleles through marker-assisted selection. In this study, an insight into the possibility of genomics-driven breeding for finger millet was gained through the identification of an association between markers and major phenotypic traits.

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Data availability The datasets used in this study are available in online repositories and the link is given under supplementary material section. The GBS quality trimmed reads were submitted to Sequence Reads Archive (SRA), BioProject PRJNA791522.

Declarations

Conflict of interest The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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