

Molecular and Phenotypic Characterization of 149 Finger Millet Accessions Using Microsatellite and Agro-Morphological Markers

B. Kalyana Babu^{1,2} · S. Sood² · P. K. Agrawal^{2,3} · C. Chandrashekara² · Arun Kumar² · Anil Kumar¹

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Abstract In the present investigation a diverse range of 149 finger millet accessions representing world collections was characterized using 19 quantitative variables and 46 genomic microsatellite markers. Wide variation was observed for the studied agro-morphological characters between exotic and Indian populations, which consisted of 60 and 89 accessions respectively. The Indian (Asian) accessions were observed to have more flag leaf sheath length, peduncle length, panicle exertion, ear head width, fingers per head and 1000-grain weight as compared to exotic populations. Highly significant and positive correlations were observed between days to 50 % flowering and days to maturity (0.914); peduncle length and panicle exertion (0.921). The first three PCA components explained 50 % of the total variation and two major groups were detected on the basis of projection of the accessions on the first two principal components. The genotypes IE7320, IE4491, GE1437, VHC3911, and VHC3898 were

found to be better parents for high photosynthetic efficiency, while GE1437, GE5192, and IE5367 were for tryptophan content. The 46 SSR markers grouped 149 genotypes into two groups viz., Indian and exotic, based on the UPGMA analysis of Power Marker V3.25 software. The clustering pattern of Indian and exotic genotypes was similar to some extent on the basis of morphological and molecular markers, however the SSR markers were more effective in explaining the admixture of genotypes. Among the SSRs UGEP65, UGEP24, UGEP60, and UGEP78 are noteworthy due to their relatively higher level of polymorphism which can be widely used for diversity analysis, and QTL mapping studies.

Keywords *Eleusine coracana* · Agro-morphological characters · Principal component analysis · DNA marker · Microsatellites

Introduction

Finger millet, *Eleusine coracana* (L.) Gaertn. ($2n = 4 \times 36$), sub-species *coracana*, belongs to the family Poaceae and the tribe Eragrostideae. It is commonly known as ragi (India), bulo (Uganda), wimbi (Swahili), and tellebun (Sudan) and is an important food crop cultivated widely in arid and semi-arid regions of the world, especially in east Africa, India and other Asian countries including Sri Lanka and China [1]. The Consultative Group on International Agricultural Research (CGIAR) has estimated that 10 % of the area under millets is with finger millet. Global area under finger millet is 3.38 million hectares with the production of 3.76 million tones [2]. It is believed that Uganda or a neighboring region is the centre of origin of *E. coracana* and it was introduced in India,

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✉ B. Kalyana Babu
kalyan_biotek@yahoo.co.in

✉ Anil Kumar
anilkumar.mbge@gmail.com

¹ Department of Molecular Biology and Genetic Engineering, College of Basic Sciences and Humanities, G.B. Pant University of Agriculture and Technology, Pantnagar 263 145, India

² Vivekananda Parvatiya Krishi Anusanthan Sansthan (VPKAS), Almora 263601, India

³ ICAR-Krishi Anusanthan Bhawan II, NASF, New Delhi, India

probably over 3000 years ago. The crop has a wide range of seasonal adaptation, and is grown in parts of Andhra Pradesh and Tamil Nadu to hills of Uttarakhand (India), Nepal, Uganda, Kenya and Ethiopia. Finger millet seeds are particularly rich in tryptophan, cystine, methionine, and total aromatic amino acids as compared to other cereals [3]. Seeds are exceptionally rich in calcium, containing about 0.34 % in whole seeds as compared with 0.01–0.06 % in most cereals [3]. Despite so many merits, finger millet has remained neglected crop as compared to major cereals such as maize, rice, and wheat.

The crop improvement programme generally involves breeding of superior and high yielding cultivars utilizing diverse germplasm resources by exploiting the variability in phenotypic characters and associations among them. Simultaneous improvement of these phenotypic characters depends on the nature and degree of association between characters [4]. There are a few reports on the phenotypic analysis and correlation among the different phenotypic characters of finger millet [5]. Initially, Mehra [6] studied rachis width, stem width, raceme width, spikelet length and glume length by metroglyph analysis in finger millet. Goud and Laxmi [7] reported the wide phenotypic and genotypic variations for tiller number, ear number and ear weight in 33 cultivars. Rao et al. [8] studied the variability among F2 generation of three crosses (Indaf 5 × GE1462, PR 202 × GE1409 and Indaf 9 × GE1409) and reported high variability, heritability and genetic advance for the traits grain yield.

Upadhyaya et al. [9] developed a core sub set of finger millet germplasm using geographical origin and data on 14 quantitative traits from a large collection of 5949 accessions. Upadhyaya et al. [10] reported the diversity pattern of 909 finger millet accessions introduced from southern and eastern African region from ICRISAT gene bank and observed large variability for days to 50 % flowering, plant height and inflorescence length. Multivariate methods are useful for characterization, evaluation and classification of plant genetic resources when a large number of accessions are to be assessed for several characters of agronomic and physiological importance [11]. The usefulness of multivariate methods for handling morphological variation in germplasm collections have been demonstrated in many crop plants including finger millet. The information generated can be useful for identifying groups of accessions that have desirable characters for crossing, for planning efficient germplasm collecting expeditions, for establishing core collections, for revealing the patterns of variation in germplasm collections and for investigating some aspects of crop evolution [12].

Genetic diversity offers opportunities to utilize various genomic resources and technologies in an effort to manipulate desirable traits. A rich diversity of landraces can be explored for their desirable traits and can be further

utilized to develop new varieties through molecular plant breeding approaches. This will pave the way towards the detection of novel and superior genotypes to meet the ever increasing demands of food grains. Salimath et al. [13] used three different DNA marker techniques, viz., RFLP (8 probe-3-enzyme combination), RAPD (18 primers) and ISSR (6 primers) to analyze the diversity of 22 accessions belonging to 5 species of *Eleusine*. Babu et al. [14] reported the diversity of 32 finger millet genotypes, using 50 RAPD markers and reported a total 529 loci of which 479 loci (91 %) were polymorphic and informative to differentiate the accessions and to do cluster analysis. Microsatellites or simple sequence repeat (SSR) markers have been useful for integrating the genetic, physical and sequence-based physical maps in plant species, and simultaneously have provided molecular breeders and geneticists with an efficient tool to link phenotypic and genotypic variation. Recently, Dida et al. [15] generated a genetic map of the finger millet genome using different types of markers like RFLP, AFLP, EST-SSRs and genomic SSR markers. Dida et al. [1] reported the population structure of 79 finger millet accessions with 45 SSR markers and identified significant difference of plant architecture and yield in Asian and African subpopulations. Till now there were no report on the extensive analysis of finger millet genotypes based on the morphological characters and microsatellite markers. In the present investigation a diverse germplasm of finger millet consisting of mini-core collection from ICRISAT and the genotypes from a wide range of seasonal adaptation in India, grown in lands to hills of Uttarakhand, India were used for genetic diversity analysis. Hence, the present study was conducted with the objectives of, (1) molecular and morphological variation among a set of 149 finger millet accessions, (2) identification of groups of accessions with similar quantitative characters using a range of multivariate statistical methods.

Material and Methods

Plant Materials

A total of 149 finger millet accessions belonging to eight countries of African continent (Zimbabwe, Kenya, Maldives, Uganda, Malawi, Senegal, Nigeria, and Zambia), two countries of south Asia (India and Nepal) and Germany were used. The seeds of finger millet obtained from different sources, which included 84 finger millet accessions from the ICRISAT mini-core gene bank, ICRISAT, Hyderabad; 31 accessions from GBPUAT, Pantnagar; and 34 accessions from VPKAS, Almora (Supplemental Table S1).

Phenotyping of Finger Millet Genotypes for Agro-Morphological Characters

The finger millet genotypes used in the present study were evaluated during rainy season of 2012, according to finger millet descriptors [16] at research farm, Hawalbagh of VPKAS, Almora. The genotypes were grown in augmented block design. The farm is located at an elevation of 1250 m above msl (mean sea level). The latitude and longitude are 29°56'N and 79°40'E respectively with a mean temperature of 16–18 °C and mean rainfall of 750 mm. Standard agronomic practices were followed to raise the crop. Observations on 19 quantitative traits were recorded in each entry on five randomly selected representative plants except for days to 50 % flowering (FLD) and days to maturity (MTD), which were recorded on the plot basis. The observations of agro-morphological characters taken during the study are given in Table 1 along with their descriptions. The 148 finger millet accessions along with checks (susceptible check-VR 708, and resistant check-RAU 8) were evaluated for blast resistance under natural conditions in the finger millet blast nursery at VPKAS, Almora, Uttarakhand, India. The blast disease severity assessment was done as per the earlier reports [17].

Quantification of Tryptophan and Protein Content

The quantification of tryptophan protocol is based on the Hopkins-Cole reaction, in which 1 molecule of glyoxylic acid and two molecules of tryptophan form a colored compound with a maximum absorption at 560 nm. The protocol used in the study is the common protocol used to determine the tryptophan content of most of the cereals especially, maize [18]. The Kjeldahl method was used for protein estimation. Protein content of finger millet genotypes was estimated from the nitrogen value which was estimated from Kjeldahl method [19].

DNA Extraction and Quantification

The seeds were grown in pots under natural conditions. DNA was isolated from 10-day-old seedlings using the protocol developed by Murray and Thompson [20]. After extraction, 1 µl of DNA each of all accessions were loaded in 0.8 % agarose gels. Uncut lambda DNA was loaded as a control to assess the quality and the quantity of DNA. Based on uncut lambda DNA standards, DNA samples were normalized to a uniform concentration (2.5 ng/µl) and used for SSR genotyping.

Table 1 List of agro-morphological characters consisting of 19 quantitative characters studied among the finger millet genotypes

S. no.	Character	Description of character
1	Days to flowering (50 %) (number)	From sowing to stage when ears have emerged from 50 % of main tillers
2	Days to maturity (number)	From sowing to stage when 50 % of main tillers have mature ears
3	Basal tiller number (number)	Number of basal tillers which bear mature ears
4	Plant height (cm)	From the ground level to tip of inflorescence at dough stage
5	Flag leaf blade length (cm)	Measured from ligule to tip of fourth blade from top at flowering
6	Flag leaf blade width (cm)	Measured across centre of fourth leaf from top at flowering
7	Flag leaf sheath length (cm)	Measured from node to ligule of flag top at flowering
8	Peduncle length (cm)	From top most node to base of the thumb finger
9	Panicle exertion (cm)	From top of leaf sheath to base of inflorescence at dough stage
10	Ear head length (cm)	From base tip of inflorescence to top of inflorescence at dough stage
11	Ear head width (cm)	Measured across the inflorescence at dough stage
12	Length of longest finger (cm)	From the base tip of longest spike from main tiller at dough stage
13	Width of longest finger (cm)	Measured across centre of longest finger at dough stage
14	Fingers per head (number)	The number of fingers present in the main ear head at dough stage was counted
15	1000 grain weight (g)	The weight of random sample of 1000 seeds from the total harvest of an accession was expressed in grams
16	Yield	Yield
17	Blast (number)	Leaf blast, neck blast and finger blast
18	Tryptophan (% of protein)	Essential amino acid
19	Protein (%)	Total crude proteins

SSR Amplification and Detection

PCR was performed in 20 μ L reaction volume containing 2 μ L 10x buffer having 15 mM $MgCl_2$, 0.2 μ M of each forward and reverse primer, 2 μ L of 2 mM dNTPs, 0.2 μ L of 1 U of *Taq* DNA polymerase (Invitrogen, USA), and about 25–50 ng of template DNA. The PCR amplification protocol was standardized for genomic SSRs, and blast specific functional markers separately. The details of the 46 genomic SSRs used in the present study were obtained from earlier studies [15] and was given in supplemental Table 2. The PCR reactions were performed in a Thermocycler (MJ Research, USA) programmed for an initial denaturation of 4 min at 95 °C followed by 35 cycles of 30 s at 95 °C, 30 s at (different annealing temperatures for different primers), 1.0 min at 72 °C, and a final extension of 10 min at 72 °C, and hold at 4 °C. The PCR products were resolved in 3.5 % super fine resolution (SFR) agarose gel (Amresco, USA). The electrophoresis was done at 100 V for 3 h at room temperature. Gels were stained with ethidium bromide and visualized using Bio Imaging System (SynGene, USA). Molecular weight of the bands was estimated using 100 bp DNA ladder as standard.

Data Analysis

The statistical analyses were performed using the JMP 2009 and statistical package SPSS (Statistical Package for

Table 2 The mean and range of the 19 quantitative traits studied among finger millet genotypes

S. no.	Character	Mean	Range
1	Days to flowering (50 %)	74.41	57–90
2	Days to maturity	110	90–123
3	Basal tiller number	2.24	1–13.5
4	Plant height (cm)	90.92	45–127.33
5	Flag leaf blade length (cm)	29.52	17.07–49.1
6	Flag leaf blade width (cm)	1.26	1–1.47
7	Flag leaf sheath length (cm)	1.26	
8	Peduncle length (cm)	19.42	3.8–30.23
9	Panicle exertion (cm)	9.93	2.17–18.3
10	Ear head length (cm)	7.00	3.9–12.57
11	Ear head width (cm)	4.78	2.64–7.67
12	Length of longest finger (cm)	5.98	2.97–11.07
13	Width of longest finger (cm)	1.29	1.1–2.1
14	Fingers per head	7.83	4–15.33
15	1000 grain weight (g)	2.31	1.39–3.12
16	Yield	12.95	
17	Blast	2.5	0–5
18	Tryptophan (% of protein)	0.85	0.6–1.4
19	Protein (%)	8.1	5.6–14

Social Science, SPSS Inc., Chicago, IL). For multi-factorial comparison, principal component analysis (PCA) was used to display the correlations between the various morphological and related parameters and their relationship with different finger millet genotypes. The data set of SSR loci on 190 accessions were used for statistical analysis using Power Marker V3.0 [21].

Results and Discussion

Evaluation of Finger Millet Genotypes for 19 Quantitative Characters

Morphological characterization is important for identification of accessions with desirable traits to employ directly as cultivars or trait donors for use in crop improvement programmes [9]. The vegetative phase was longer in the genotype IE4816 (90 days) followed by IE2312, IE5201 and IE3699 (89 days each). However, it was lowest in the genotype VR708 (57 days) (Table 2). The average plant height observed was 90.92 cm with a range from 45 to 127.33 cm. Early flowering accessions were reported in finger millet [22], pearl millet [23], and chickpea [24]. The minimum number of days to maturity observed was 90 days, however maximum number of days to maturity observed was 123 with a mean of 110 days. The variation observed for quantitative traits was similar to the earlier reports in finger millet germplasm [10]. Upadhyaya et al. [9], developed core subset of finger millet germplasm using 14 quantitative traits reported similar mean values for flag leaf blade width, flag leaf sheath length, width of longest finger, length of longest finger, peduncle length and panicle exertion, however they observed higher values for days to 50 % flowering, days to maturity and basal tiller number. This variation may be due to environmental conditions and number of genotypes included in the study. The mean values observed in the study for days to 50 % flowering, fingers per head and 1000 grain weight were in corroboration to Dhanalakshmi et al. [25], however slight variation was observed in case of plant height. The evaluation material was similar but the environment was different, this indicates the importance of evaluation of germplasm in different environments for its judicious use in crop improvement. Most of the exotic genotypes and ICRISAT mini core collection showed highly resistant to moderately resistant reaction to neck blast disease, except the genotype from Nepal (IE6082) which showed susceptible reaction. The susceptibility of the IE6082 genotype to neck blast may be due to the proximity of Nepal to NW Himalayan region and there might be germplasm exchange between Nepal and the NW Himalayan region of India. The genotypes VHC3917, VHC3939, VHC3870, and VHC3970

which belonged to NW Himalayan region of India were found highly susceptible to finger blast disease. The susceptibility reaction of these genotypes may be due to earliness in maturity, coincidence of disease favoring conditions and reproductive phase. However, most of the medium and late duration genotypes escape the disease even if they are susceptible to disease. Among the genotypes from NW Himalayan region of India, the accessions, VHC3997, VL324, and VHC3996 were highly resistant to finger blast. However, exotic genotypes were found to be resistant to finger blast like neck blast disease, which was well supported by the earlier studies [26]. The protein content (average of three replications) of the selected 149 finger millet genotypes varied from 5.6 to 14 % with an average of 8.1 %. Similar results were also reported earlier for protein content in finger millet genotypes [27].

Wide variation was observed for the studied agro-morphological characters among the exotic and Indian populations except for flag leaf width and width of longest inflorescence. Considering the existence of a clear population structure in the germplasm analyzed, it is not surprising that distinct differences can be found in agro-morphological traits between the two populations. The overall trend observed was that Indian (Asian) accessions were smaller in stature with smaller flag leaves, less basal tillers, early flowering and maturity and smaller ear heads. These results indicated that the African genotypes were adapted well along with Indian accessions, since Indian genotypes were largely derived from the African germplasm. Similar results have been obtained by Dida et al. [1] where they observed that Asian accessions were smaller in stature having smaller flag leaves. However, the results were contradictory for traits like basal tiller number and fingers per head. Exotic finger millet genotypes had good tillering ability than Indian finger millet genotypes. Similar results for high tiller number in east African genotypes were found by Bharathi [28].

Correlation Coefficient Between Quantitative Traits

Correlation coefficient helps to estimate the degree of association among the different agro-morphological characters using phenotypic values. In the present study, days to 50 % flowering was positively correlated with days to maturity, plant height, flag leaf length and width, ear head length and length of longest finger. Bharathi [28] also reported a significant positive association between days to 50 % flowering, ear head length and length of longest finger. The plant height was positively correlated with days to 50 % flowering, flag leaf length and width, peduncle length, panicle exertion, ear head length and width and yield. Sivagurunathan [29] reported a positive correlation of plant height with ear head length, panicle exertion and

grain yield. Highly significant and positive correlations were observed between days to 50 % flowering and days to maturity (0.914); peduncle length and panicle exertion (0.921). These correlation studies will help in formulating a selection index of traits like days to flowering, flag leaf sheath length, peduncle length, length of longest finger and finger blast in the finger millet breeding programmes instead of considering both the parameters with high correlation value. This will reduce the time and space in breeding programmes. Dhanalakshmi et al. [26] also found significant and positive correlation between days to maturity and days to 50 % flowering (0.604). The highly significant correlation between peduncle length and panicle exertion will lead to low number of racemes in the inflorescence and there will be in-turn reduction in the grain yield and hence high value should be avoided in the yield enhancement programmes. Highly significant and negative correlation was observed between tryptophan and protein content (-0.627) indicating that higher protein value will not end up in high tryptophan content. Neck blast was found to have significant and positive correlation with finger blast (0.612**), but a poor correlation was observed with leaf blast (0.08**). The correlation coefficient results were similar to the earlier reports [27, 30].

The evaluation data provides a valuable opportunity to assess relationship among traits in order to test the similarity between different groups [31]. This will simplify work and save resources. Of the 210 character associations estimated, four associations namely peduncle length versus panicle exertion (0.921), flag leaf sheath length versus peduncle length (0.819) and length of longest finger versus ear head length (0.910) had reasonably high estimates indicating that in future characterization of finger millet germplasm peduncle length and ear head length data could be used instead of tedious observations like panicle exertion and length of longest finger. Upadhyaya et al. [9] also observed strong association of peduncle length and panicle exertion in finger millet and emphasized on taking observation on easy trait, panicle length than panicle exertion.

Principal Component Analysis (PCA)

Interrelationships among the different parameters were studied using PCA. The first three PCA components provided a reasonable summary of the data and explained 50 % of the total variation and subsequent components contributed 8 % or less. The percent variation explained by PCs in present study is lower than earlier reports [28, 32]. The first principal component (PC1), was the most important which explained 23.7 % of the total variance (Fig. 1). The traits peduncle length, flag leaf sheath length, fingers per head, leaf blast, finger blast, 1000 grain weight, neck blast and protein had largest negative loadings. The

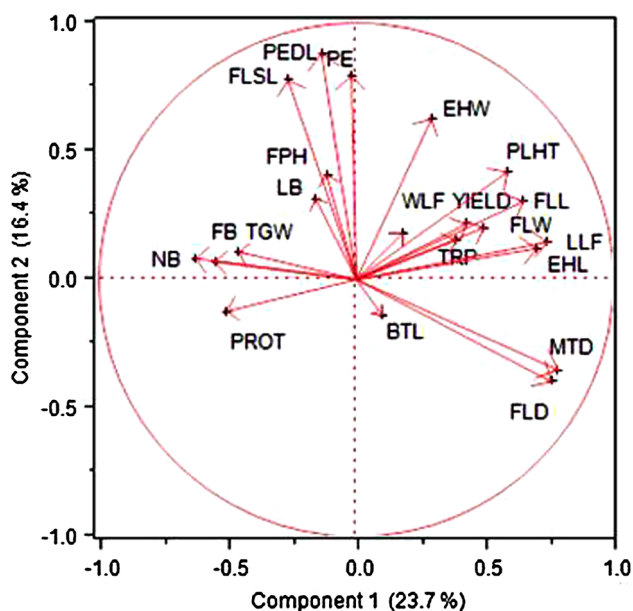
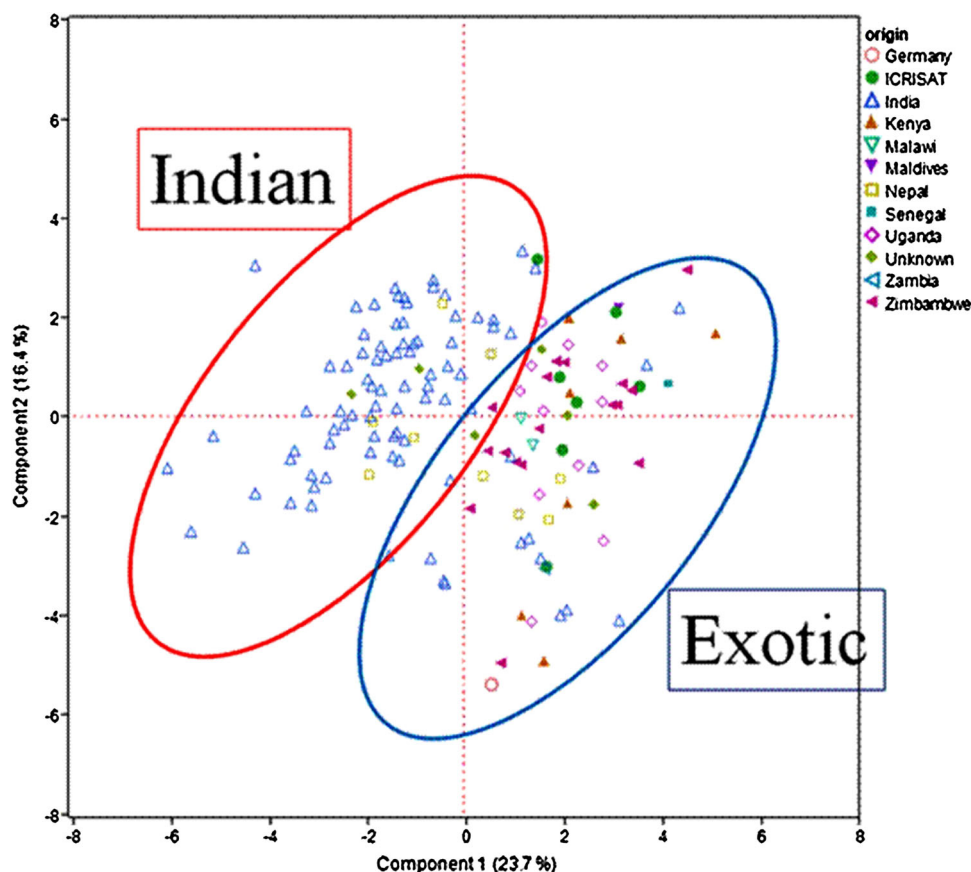


Fig. 1 Loading plot of PC1–PC2 for 149 finger millet genotypes (*FLD* days to 50 % flowering, *MTD* days to maturity, *LLF* length of longest finger, *EHL* ear head length, *FLL* flag leaf blade length, *BTL* number of basal tillers, *PE* panicle exertion, *FPH* fingers per head, *EHW* ear head width, *PEDL* peduncle length, *FLSL* flag leaf sheath length, *NB* neck blast, *FB* finger blast, *TGW* thousand grain weight, *LB* leaf blast, *WLF* width of longest finger, *PLHT* plant height, *FLW* flag leaf width, *TRP* tryptophan)

Fig. 2 Score plot of PC1–PC2 for 149 finger millet genotypes



second PC explained an additional 16.4 % of the total variance and was attributed to positive loadings of all of the traits except protein, basal tiller number, days to 50 % flowering and days to maturity. The second component basically identified the accessions with high values for panicle exertion, peduncle length, flag leaf sheath length, ear head width and plant height. Two major groups were detected on the basis of projection of the accessions on the first 2 PCs (Fig. 2). The first group (G1) consisted of almost all the Indian accessions along with unknown accessions and three accessions from Nepal, were on top and bottom left of the plot. The second group (G2) consisted of finger millet accessions from exotic origin, which belonged to Kenya, Malawi, Maldives, Senegal, Uganda, Zambia, Nepal and Zimbabwe. They were on the top and bottom right of the plot. When more than three variables have been measured, visualization of the data by various plotting systems is then possible by PCA [33]. Therefore, PCA indicated relationships among groups of variables in a data set and show relationships that might exist between objects. The PCA revealed that Indian genotypes were better for the quantitative traits such as peduncle length, flag leaf sheath length, fingers per head, leaf blast, finger blast, 1000 grain weight, days to 50 % flowering, maturity, neck blast and protein, while the exotic genotypes were

better for ear head width, plant height, width of longest finger, grain yield, flag leaf blade length, flag leaf blade width, tryptophan content, length of longest finger, ear head length and number of basal tillers. Scatter plot of PCA1 and PCA2 showed overlapping of accessions of Indian and exotic origin genotypes. A possible explanation may be introgression of genes from exotic to Indian and vice versa.

Phylogenetic Analysis of Finger Millet Genotypes

Two way cluster analysis separated the accessions as well as traits into two major groups. The Group 'A' consisted of 78 genotypes mostly from India, with a few exceptions. The Group B comprised of 71 genotypes of exotic origin clustered together with a few Indian genotypes (GPU28, VHC3997, GE4404 etc.). The results of the cluster analysis were in congruence with the PCA analysis, where the PC1 and PC2 also resulted in two major groups. The cluster analysis resulted in grouping of the selected finger millet genotypes based on the geographic origin with a few exceptions. The group A was further divided into two sub clusters A1 and A2 (Supplemental figure S3). The sub cluster A1 consisted of genotypes mostly belonged to the Indian plains except VHC3870, VHC3970, and VHC3984 which belonged to the NW Himalayan region of India. However, A2 sub cluster consisted of genotypes from NW Himalayan region of India with a few exceptions like RAU8 and GE496. This type of mixture of genotypes may be due to the fact that there might be germplasm exchange between different places of India for better varieties of finger millet. The major cluster B was further divided into three sub clusters B1, B2, and B3. The cluster B3 consisted of all the exotic genotypes except VHC3997, GE763, GE4404, GPU48, GE554 and GE4692 which were of Indian origin. The morphological traits were also grouped into two major clusters where leaf blast, fingers per head, basal tiller number, width of longest finger, finger blast, neck blast, protein, 1000 grain weight, flag leaf sheath length, peduncle length and panicle exertion were under group 1, whereas tryptophan, plant height, yield, flag leaf length, days to 50 % flowering, days to maturity, ear head length, length of longest finger and ear head width were under group 2. The phylogenetic clustering showed that Indian accessions were best suitable to the agro-morphological characters under group 1, whereas exotic accessions were best suitable for agro-morphological characters under group 2.

Selection of Promising Accessions

The core germplasm evaluation helped in identifying the promising trait donors which could be efficiently used in

breeding programmes for the improvement of the crop. The days to 50 % flowering and days to maturity were kept lowest for selection of genotypes suitable for the NW Himalayan region of India, where mostly short duration and early flowering accessions are preferred because of climatic conditions. In the present study, the genotypes VR708, IE501, IE1621, IE3104, VHC3903, and VL201 found to be early maturing accessions which could be suitable to the NW Himalayan region of India. The major breeding programmes at NW Himalayan region of India also focused on more plant height of finger millet genotypes for the need of fodder purpose cultivars along with short duration. The genotypes IE6473, IE3700, IE5066, IE6421, and IE2430 which had high plant height could be the best donors in high fodder improvement programmes of finger millet (Table 3). Yield is an important parameter, which was influenced by several parameters like ear head length, ear head width, and length of longest finger. Higher values of these traits result in higher yield, and in the present study, the genotypes IE6514, IE3712, IE4028, VHC3997, VL324, and VL149 were selected as best donors when compared to other genotypes for better yield and their further use in the breeding programmes. The finger millet crop is considered as nutria-cereal and in the present study the authors evaluated tryptophan, an essential amino acid of proteins and total proteins. Based on the tryptophan values the accessions GE1437, GE5192, IE6240, GE2136, and IE5367 were found to have high tryptophan content and can be used as donors. Among the genotypes from NW Himalayan region, VHC3917, VHC393, VHC3870 and VHC3970 were found to be highly resistant to finger and neck blast and can be ideal candidates for resistance breeding in the NW Himalayan region. Most of the exotic genotypes were found highly resistant to neck and finger blast, but need to be tested for their suitability as donors in the breeding programmes especially in NW Himalayan region of India.

Microsatellite Analysis

The genomic DNA of global collection consisting of 149 finger millet genotypes which were amplified using 46 genomic SSRs. These 46 SSRs were spread across the chromosomes of finger millet genome. The gel pattern of the finger millet accessions using the SSR marker UGEP24 was given in Fig. 3. The 46 SSRs yielded 90 scorable alleles, of which 65 were found polymorphic. A total of 21 (46 %), out of the 46 SSR loci were found polymorphic and the remaining 25 (54 %) were monomorphic SSR loci. The percentage of polymorphism obtained in the study was lesser than earlier studies [28], however more than that of barley [34]. Normally in inbreeding species, the level of polymorphism is expected to be generally lower than in out

Table 3 The promising trait donors of finger millet germplasm for 19 quantitative traits

S. no.	Trait	Promising accessions
1	Days to 50 % flowering (≤ 60 days)	VR708, IE501, IE1621, IE3104, VHC3903, VL201
2	Days to maturity (≤ 94 days)	VR708, IE1621, VL201, IE501, VHC3903
3	Basal tiller number (≥ 6)	IE3077, IE2217, IE4491, IE4797, IE2437
4	Plant height (≥ 124 cm)	IE6473, IE3700, IE5066, IE6421, IE2430
5	Flag leaf blade length (> 43 cm)	IE7320, IE4491, IE3791, IE4797, GE1437
6	Flag leaf blade width (≥ 1.37 cm)	GE1437, IE4491, IE4497, GE4811, VHC3996, VHC3898
7	Flag leaf sheath length (≥ 12.63 cm)	VHC3911, IE4797, VHC3898, VHC3881, VHC3893, GE384, GE128
8	Peduncle length (≥ 26.50 cm)	IE2821, IE2430, IE3973, GE1146, IE4491
9	Panicle exertion (≥ 15.23 cm)	IE2821, IE2430, GE1146, IE4491, IE3795
10	Ear head length (≥ 11.0 cm)	VHC3908, IE4491, IE7320, GE4692, GE514, IE4565
11	Ear head width (≥ 6.70 cm)	VHC3908, IE4673, VL149, VHC3991, RAU8, GE496
12	Length of longest finger (≥ 9.50 cm)	IE4491, IE4565, VHC3991, GE514, VHC3908, IE7320
13	Width of longest finger (≥ 1.40 cm)	IE7320, VHC3870, IE4671, IE5306, GE4440, RAU8
14	Fingers per head (≥ 12.67)	VHC3996, VHC3898, VHC3917, VHC3962, VHC3881, VHC3972
15	1000 grain weight (≥ 3.12)	GE390, GE3147, GE1537, GE619, GE514, VHC3908, RAU8
16	Yield (> 23.0)	IE6514, IE3712, IE4028, VHC3997, VL324, VL149
17	Blast	VHC3997, VHC3930, VL324, VHC3996
18	Tryptophan (> 1.20 %)	GE1437, GE5192, IE6240, GE2136, IE5367
19	Protein (> 11 %)	GE1680, GE1621, GE1537, GE1235, GE1240, GE1583, GE619

crossing species [35]. This type of low level of polymorphism may be due to the fact that the finger millet crop is highly self pollinated. The 21 polymorphic loci yielded 55 scorable alleles with a mean of 2.9 alleles per locus. The allele number per SSR locus in this study was lesser to the earlier studies in finger millet as 6.42 alleles per locus [1]. The polymorphism information content (PIC) demonstrates the informativeness of the SSR loci and their potential to detect differences among the genotypes based on their genetic relationships. The PIC values of all the polymorphic loci across the 149 finger millet genotypes varied from 0.292 to 0.703 at an average of 0.442. Similar results have been obtained by Babu et al. [36] where they reported a slightly more PIC at the range of 0.19–0.67 among a set of 52 finger millet genotypes. Recently Bharathi [28] also used genomic SSRs for the diversity analysis and found PIC range from 0.196 to 0.834. These results showed that the results obtained in the study were largely in congruence with the earlier studies. In-contrast, very less PIC values were obtained by Nirgude et al. [27] where they found PIC range from 0.023 to 0.256 based on the EST-SSRs which may be due to less number of SSRs used in their study. Out of the 21 polymorphic SSR markers, six SSRs showed highest PIC value of more than 5.00 and eight loci were found to have more PIC value than the average value. Among the SSR loci, UGEP65, UGEP24, UGEP60, and UGEP78 are noteworthy due to their relatively higher level of polymorphism. The SSR loci which had more PIC value also generated more number of alleles. In the present study,

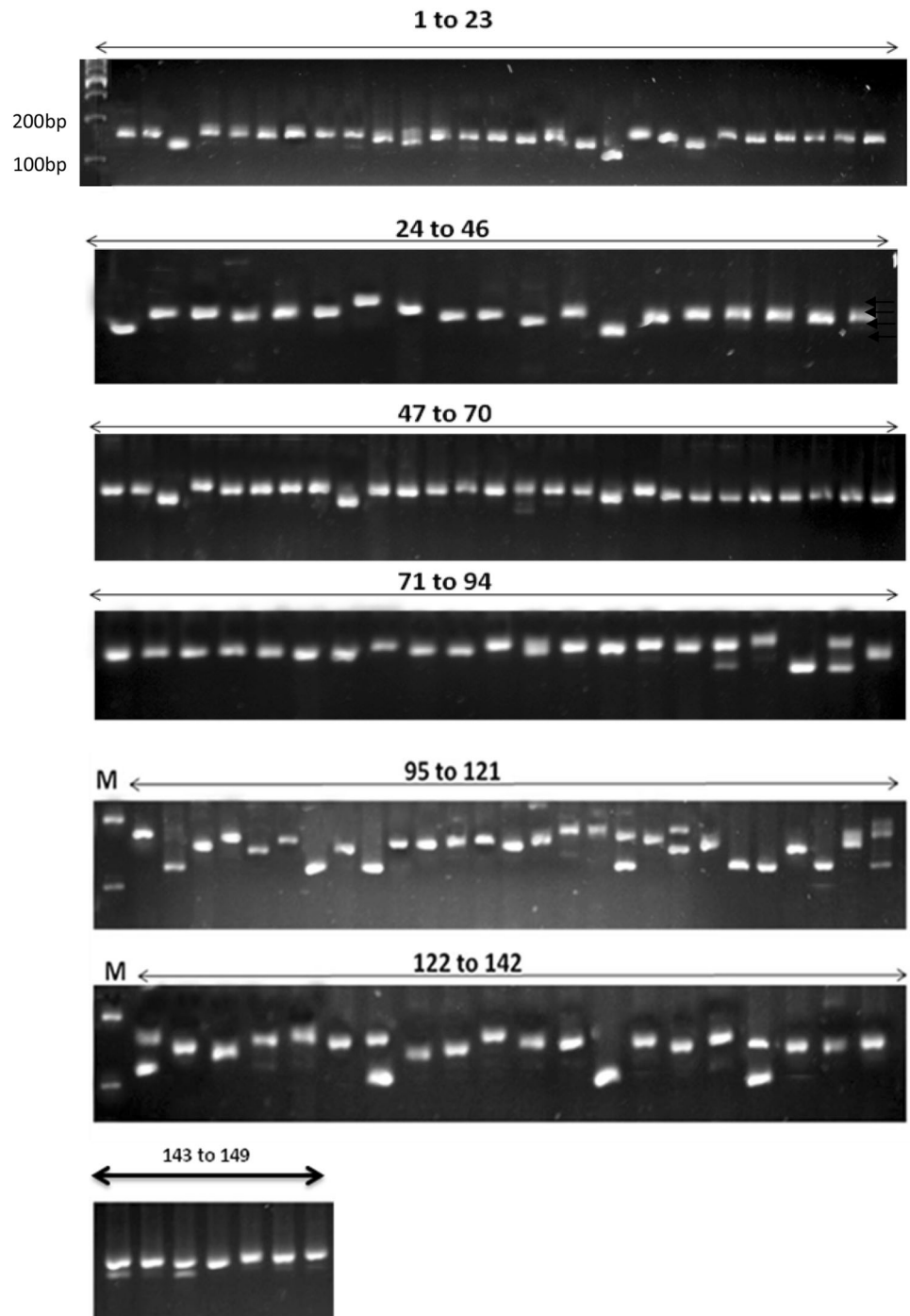
close proportionate relationship between the number of alleles and the PIC values of SSR markers was observed. For example UGEP24 and UGEP65 were found to have high PIC values and also generated more number of alleles.

Among the polymorphic SSR loci, 20 loci had dimeric repeat motifs, whereas one locus UGEP108 had trimeric repeat motif (CTG). The SSR loci (UGEP60, UGEP78, UGEP10, and UGEP24) containing the GA repeat motifs showed highest PIC values than any other repeats. The SSR loci which got more PIC value than 5.00 had GA dimer repeat motif except UGEP65 which has CT dimer motif. All the loci containing CT dimer repeat motif had lower PIC values except UGEP65. These results showed that the SSR loci containing the GA repeat motif had more PIC, more allele number, highest gene diversity and lowest heterozygosity, hence they can be used in molecular breeding programmes of finger millet for higher polymorphism studies.

Diversity Analysis of Finger Millet Genotypes Based on Genomic SSR Loci

Genetic diversity is the basic requirement for the crop improvement programme. Knowledge of germplasm diversity has significant impact on the crop improvement. Efficient use of conserved bio-diversity requires information about the degree and distribution of genetic diversity. Assessment of diversity using molecular markers would provide an opportunity to select appropriate parents for

Fig. 3 The banding pattern of 149 finger millet genotypes with the genomic SSR locus UGEP24 (for labels, please refer to table S1)



crop improvement with higher precision. The importance of increased use of genetic resources in enhancing genetic potential of crops alleviating biotic and abiotic stresses and broadening genetic base of crop has been very well recognized [37]. Finger millet, like other millets, has a narrow genetic base in spite of the large collection of germplasm and globally active genetic enhancement programme. With the discovery of a large number of genomic SSR markers [1], it is now possible to conduct extensive molecular

diversity in finger millet for identifying genetically diverse germplasm with beneficial traits for use in crop improvement programmes.

Molecular markers have been applied in quantification of genetic diversity, genotype identification, gene mapping, association mapping and marker assisted selection (MAS). Improvement of finger millet breeding requires identification of highly diverse germplasm and highly polymorphic molecular markers, and their effective utilization in the

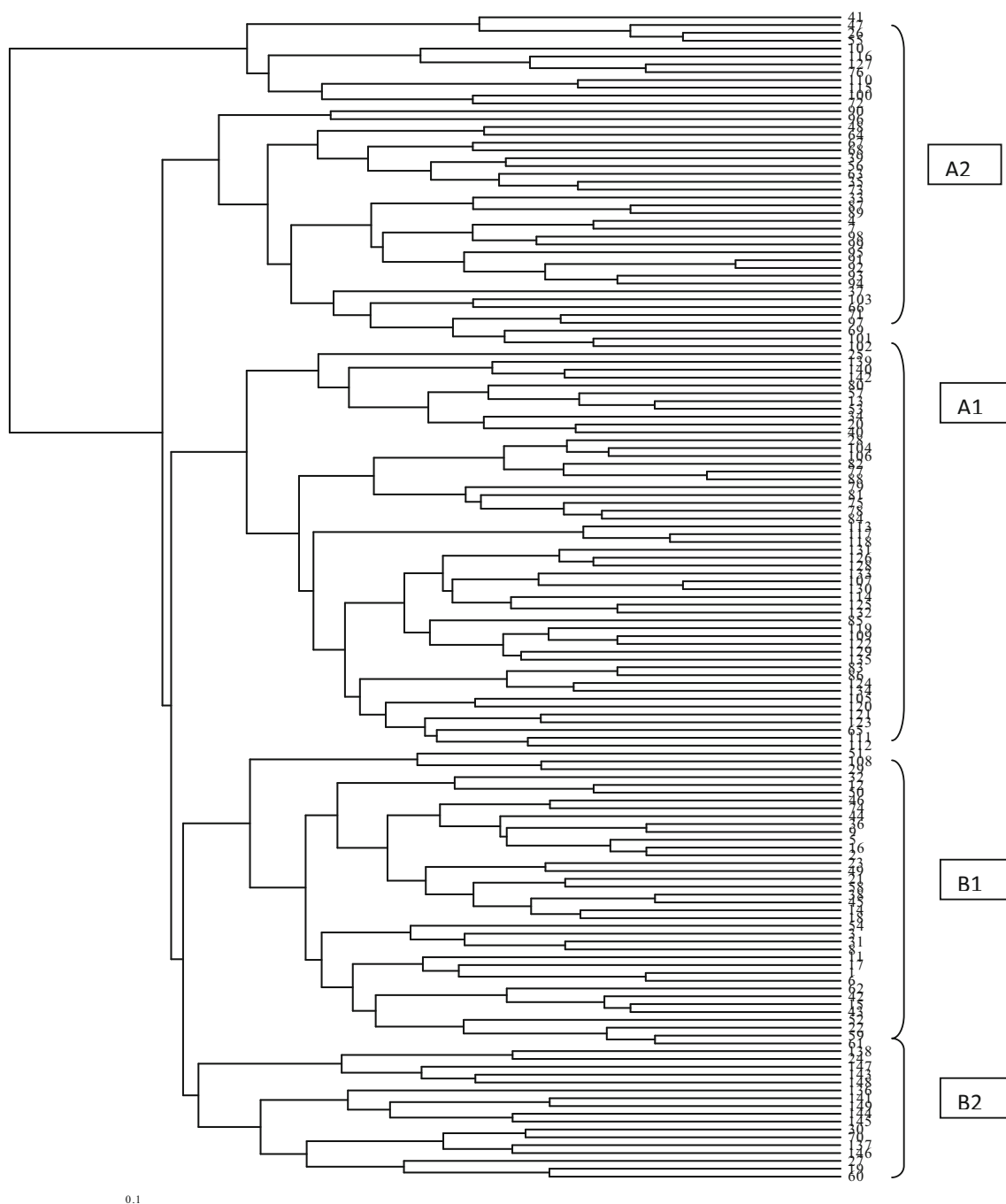


Fig. 4 The dendrogram generated by Power Marker software using genomic SSRs of finger millet (for labels, please refer to table S1)

identification of quantitative trait loci (QTLs) for agronomically important traits. In the present study, the genetic diversity analysis among the global collection of 149 finger millet genotypes were done using 46 genomic SSRs. The SSR loci grouped the 149 finger millet genotypes into two major clusters (A and B) based on the UPGMA analysis of Power marker V3.25 software (Fig. 4). The major cluster A comprised broadly all the Indian genotypes, whereas cluster B comprised of exotic genotypes. These groupings,

in most instances, revealed evidence of associations related to their geographical origin. Similar type of results have been obtained in the earlier reports [28]. Bharathi [28] analyzed a set of composite collection of finger millet genotypes based on the genomic SSRs and found clear patterns of genetic diversity among the African and Asian accessions based on similarity index of SSR allelic patterns. Dida et al. [1] used 45 SSR markers for diversity and population structure analysis of *E. coracana* germplasm

and found three groups of Africana, African coracana and Asian coracana lines. These results indicated that ability of SSR markers in differentiating the finger millet germplasm based on their geographical origin. The average gene diversity existing among all the inbred lines were relatively high (53 %), indicating existence of high levels of polymorphism among the finger millet. These results are in close agreement with the findings reported among the cereal crops like maize inbreds using a SSR marker system [38].

The cluster A comprised of 90 finger millet genotypes, where most of the genotypes belonged to India. The major cluster A is further divided into two sub clusters A1 and A2. The sub cluster A1 consisted of 43 finger millet genotypes, whereas A2 sub cluster comprised 47 genotypes. The sub cluster A2 consisted of 47 finger millet genotypes which were mostly of Indian origin and were clustered with a few African finger millet accessions. These results showed that there may be involvement of germplasm exchange between them for breeding the superior genotypes adopted for India. The sub cluster A2 consisted of a few exotic genotypes from south Asian and African countries viz, two genotypes from Nepal (IE2821 and IE5817), three genotypes from Zimbabwe (IE6350, IE4565 and IE4545), and three from Uganda (GE4692, IE6473 and IE6421). This fits with the hypothesis that finger millet was first domesticated in the African highlands, and from there it was introduced to India. Similar observations have been reported by Dida et al. [1]. They reported majority of the accessions of Asian origin to group together and to act as sister group of the accessions that originated mostly from African lowland countries. Tight clustering of the Zimbabwe genotype IE6350 with the Indian genotypes (RAU8, GPU28) showed that during domestication, allele transferring might have taken place. The Indian-African hybrid GPU28 was grouped with RAU8 and KM252. The genotype RAU8 was of Indian origin, whereas origin of KM252 is unknown. The hybrid GPU28 might have developed from crossing between the Indian and African lines.

The cluster B comprised of 59 finger millet genotypes mostly of exotic origin from eight countries of African continent and south Asian region. The cluster B is further divided into two sub clusters B1 and B2. The sub cluster B1 comprised of 42 genotypes, all being exotic genotypes with a few exceptions (IE3104, IE3475 and IE4671). All the exotic genotypes spread randomly across the cluster B1 except the genotypes from Zimbabwe. The above three genotypes were closely clustered with two Nepal genotypes viz., IE6165 and IE6059. This showed that domestication of finger millet germplasm might have moved from Africa to India and then to Nepal. The sub cluster B2 contains 17 finger millet genotypes which comprised of both Indian and exotic genotypes. This showed that though they were clustered with

exotic genotypes, but formed separate cluster, since the geographic alleles were more influencing than the parentage. This type of clustering of Asian and African genotypes was also observed by the Bharathi [29]. Similarly, Nepal genotypes (IE2821 and IE5817) were clustered with Indian genotypes belonging to NW Himalayan region of India, as the region is very close to Nepal. This may be due to the fact that there was exchange of Indian genetic material between these two geographical origins for different purposes of breeding programmes. Similarly, the genotype GPU48 which is an Indo-African hybrid. It is closely clustered with GE496 (Indian origin), and GE5192 (Uganda origin). So, the present results are clearly in agreement with the parentage and geographical origin of the genotypes.

The results obtained from morphological and SSR based characterization were similar to some extent for diversity analysis based on the geographic origins. However, molecular markers are more specific in evaluating the admixture of genome between the Indian and exotic germplasm. Morphological evaluation resulted in grouping accessions mostly based on the source of collection and their geographical locations. However, microsatellite based characterization was able to differentiate not only the geographic factors, but also explained the admixture of germplasm among the genotypes which may be due to germplasm exchange in the breeding programmes of finger millet.

Conclusion

The conclusions of the present investigation showed that there is a lot of variation which existed among the 19 quantitative traits evaluated. The study identified best donor or parent genotypes for different quantitative traits based on correlation and multivariate analysis studies for their further use in the finger millet breeding programmes. The clustering pattern of the Indian and exotic genotypes was similar to some extent by both morphological and molecular markers, however the SSR markers were more effective in the germplasm evaluation for explaining the admixture of genotypes. Among the SSR loci, UGEP65, UGEP24, UGEP60, and UGEP78 are noteworthy due to their relatively higher level of polymorphism which can be widely used for diversity analysis, and QTL mapping studies.

Future Perspectives

The identified polymorphic markers can be effectively used in genetic diversity studies of finger millet germplasm. The identified accessions for specific traits can be used in the future breeding programmes for blast resistance, higher yields, fodder improvement and for nutritional quality.

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Compliance with Ethical Standards

Conflict of interest The authors declare that there is no conflict of interest.

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