

## Genetic diversity and variability in Foxtail millet [*Setaria italica* (L.)] germplasm based on morphological traits

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### Abstract

Fifty one accessions of foxtail millet (*Setaria italica* (L.) P. Beauv.), constituting a national elite germplasm collection were evaluated for morphological diversity based on nine quantitative traits viz., plant height, number of basal tillers, days to flowering, flag leaf length, flag leaf width, petiole length, inflorescence length, thousand seed weight and single plant yield. The traits showed a continuous distribution, however number of basal tillers, and single plant yield exhibited skewness in positive directions. Considerable variability was observed among the genotypes for all the traits. Among these, grain yield per plant exhibited the maximum variation with a CV of 22.5%, while days to fifty per cent flowering and plant height exhibited the least variation with a CV 7.2% and 9.3% respectively. PCA analysis revealed that the first four components in the PCA results contributed to a maximum of 70 per cent of the variability, the contributions from PC1, PC2, PC3 and PC4 being 30.1%, 14.2%, 13.8%, and 11.4%, respectively. The first two components were a measure of vegetative and inflorescence characters, while the third component was a measure of thousand seed weight. Cluster analysis based on the nine morphological traits assorted the 51 genotypes into four main clusters. Dendrogram based on hierarchical clustering grouped the genotypes based on their morphological traits, however the grouping of genotypes did not correspond with their geographic origin. Plant height, number of tillers, days to fifty per cent flowering, grain yield per plant, and 1000 seed weight were found to be the most important traits in distinguishing the major clusters of foxtail millet genotypes at the morphological level.

**Key Words:** Foxtail millet, Genetic Diversity, PCA, Cluster analysis

### Introduction

Foxtail millet [*Setaria italica* (L.) P. Beauv.] is a crop of the Neolithic era, known to have been domesticated more than 8700 years ago in China. Over years of domestication, its cultivation has spread throughout Asia and Europe (Naciri *et al.*, 1992; Goron and Rhizada, 2015). It ranks second in the world with respect to total millet production and serves as an important staple food for millions of people in Southern Europe and Asia, particularly in China and India (Marathee 1993, Baltensperger, 1996). Taxonomically, foxtail millet comprises of two subspecies namely *Setaria viridis* and *Setaria italica*. The cultivated foxtail millet belongs to *Setaria italica* which comprises of three races and ten subraces (Jusuf and Pernes, 1985). Due to its geographically wide spread adaptation, foxtail millet exhibits a wide range of genetic diversity. Collection, conservation and characterization of these genetic resources are a prerequisite for the genetic improvement of foxtail millet. The Chinese National

Gene Bank (CNGB) in China, International Crops Research Institute for the Semi-Arid Tropics (ICRISAT) and National Bureau of Plant Genetic Resources (NBPGR) in India, National Institute of Agrobiological Sciences (NIAS) in Japan, and Plant Genetic Resources Conservation Unit of USDA in USA are some important institutions maintaining the world's foxtail millet collections. These germplasm collections are being characterized morphologically and some core and mini core collections have been assembled (Upadhyaya *et al.*, 2008, 2011; Li *et al.*, 1998). Exploiting the germplasm for trait improvement depends on the extent of genetic variability present in the gene pool. Genetic variability in a germplasm can be assessed using morphological traits and molecular markers. Although influenced by environment, morphological traits depict the diversity visually, and also influence the yield potential of genotypes and their adaptations' to stress environments. The influence of these traits on yield is variable in direction and magnitude, and has served as selection indicators in identifying

genotypes with traits of interest. Multivariate analysis such as principal component analysis (PCA) and cluster analysis serve as potential tools in evaluating the phenotypic diversity, identifying genetically distant clusters of genotypes and selecting important traits' contributing to the total variation in the germplasm. These analyses provide information that could help in better selection of parental genotypes with specific traits and in devising breeding strategies for trait improvement. However, very little information on these aspects is available in foxtail millet (Sandhu *et al.*, 1974; Nagarajan and Prasad, 1980; Islam *et al.*, 1990). Hence, the present study was aimed to characterize the level of variability present in a national elite germplasm collection of foxtail millet for utilization in breeding programmes.

### Materials and Methods

Fifty one national elite foxtail millet accessions including checks obtained from the All India Coordinated Small Millet Improvement Project (AICSMIP) unit, Bengaluru were evaluated in the present study. These accessions represented diverse geographic regions of India (Table 1). The trial was conducted during *Kharif*, 2014 at Millets Breeding Station (MBS), Tamil Nadu Agricultural University, Coimbatore, India, which is situated at about 11°N latitude and 77°E longitude at an altitude of 427 metres above MSL. The average annual rainfall is around 700 mm. The trial was laid out in an augmented block design I (Federer and Raghavarao, 1975) with two checks *viz.*, CO(Te7) and SiA326 in each block. Each accession was grown in a single row of three meters length with a spacing of 30 cm between rows and 10 cm between plants for morphological characterization and evaluation. The recommended agronomic packages of practices were followed during the experimental period. Nine morphological traits were recorded on five randomly selected plants of each accession as per the standard descriptors described for *Setaria italica* (IBPGR, 1985). The nine traits measured are as follows: plant height (PH) [measured in cm from ground level to the tip of the inflorescence at dough stage], number of basal tillers per plant (NBT) [measured as number of tillers at ground level or from the basal nodes], days to 50% flowering (DFF) [measured as number of days from sowing to the stage when the ears have emerged on 50% of the main tillers], flag leaf length (FLL) [measured in cm from ligule to flag leaf tip at flowering stage], flag leaf width (FLW) [measured in cm across the centre of the flag leaf at flowering], peduncle length (PL) [measured in cm from top most node to the base of the inflorescence], inflorescence

length (IL) [measured in cm from base to the tip of the ear on the main tiller at dough stage], 1000 seed weight (SW) [measured as weight of 1000 seeds (g) sampled randomly from the total harvest of an accession] and single plant yield (SPY) [measured in g as the mean grain yield per plant based on five random plants]. The data were subjected to the following statistical analysis. Basic descriptive statistics were obtained using the statistical package SPSS 16.0 version. The test statistic for skewness and kurtosis were worked out using the formulae adapted by Cramer, 1997. Frequency distribution and PCA was performed using the statistical package SPSS 16.0 version. The Wards method of hierarchical clustering technique (Ward, 1963) was employed to group the 51 accessions based on similarity matrix as implemented in Darwin software version 5 (Dissimilarity Analysis and Representation for windows) V.5.0.158 (Perrier and Jacquemoud-Collet, 2006) (<http://darwin.cirad.fr/darwin>).

### Results and Discussion

The basic statistical measures *viz.*, maximum, minimum, mean, variance, standard deviation (SD), coefficient of variation (CV), skewness and kurtosis coefficients for the measured traits are presented in Table 2. The traits showed a continuous variation and the frequency distribution of the nine traits is presented in Figure 1.

Plant height ranged from 103.96 to 176.18cm. The number of basal tillers ranged from 3 to 6.2, with ISe1 recording the maximum number of basal tillers. The genotype ISe144K had taken the most number of days (60 days) to attain 50 per cent flowering, while ISe1332 attained 50 per cent flowering within a short span of 40 days. Among 51 genotypes, 39 genotypes attained 50% flowering within 48-55 days. On an average the flag leaf length, flag leaf width, peduncle length and inflorescence length in the elite germplasm collection was 28.04cm, 2.04cm, 23.69cm and 18.85cm respectively. Single plant yields ranged from 13.0 to 34.0g. The mean thousand grain weight in the germplasm collection was 2.92g.

Among the nine traits, the largest variation was observed for single plant yield with the coefficient of variation amounting to 22.55 per cent, followed by number of basal tillers (14.6%), peduncle length (13.98%), inflorescence length (13.49%) and flag leaf width (12.12%). Least coefficient of variation of 7.21 per cent was observed for days to fifty percent flowering. While cataloguing the foxtail millet germplasm, similar results were obtained by Gowda

*et al.* (2002) and Islam *et al.* (1990). In an assembled collection of 1482 accessions, large variations were recorded for seed yield per plant (g) (39.99%) followed by number of basal tillers (36.24%), which is an important yield contributing character. The variation for inflorescence and flag leaf traits ranged from 15.82 to 19.92 per cent. However, days to fifty percent flowering showed the least variation with a CV of 7.58 per cent (Gowda *et al.*, 2002). Similarly, Islam *et al.*, (1990) also reported that variability was higher for grain yield and number of tillers, in a germplasm evaluation comprising of 78 accessions.

Although the traits showed continuous variation, none of the traits showed a perfect symmetrical normal distribution as evidenced from the skewness and kurtosis parameters. Analysis of quantitative traits using skewness and kurtosis parameters provides information about the quantum and nature of gene action controlling the traits (Fisher *et al.*, 1932; Robson, 1956). Skewness is an indicator used in distribution analysis as a sign of asymmetry and deviation from the normal distribution pattern. Positive skewness means that the asymmetric distribution is with a longer right tail, while negative skewness indicates the asymmetric distribution with a longer left tail (Herman and Theis, 2007). In this study, traits such as number of basal tillers, peduncle length, single plant yield and thousand seed weight exhibited a right skewed distribution. Traits such as plant height, days to fifty percent flowering, flag leaf length, flag leaf width and inflorescence length exhibited a left skewed distribution, which indicates that the extreme genotypes were to the left of the mean and most values were concentrated on the right of the mean (Table 2). Among these nine traits, the two traits which exhibited the largest variation *viz.*, number of basal tillers and single plant yield were significantly skewed in their distribution. Gowda *et al.*, (2002) reported right skewed distribution for traits such as number of basal tillers, flag leaf length, flag leaf width, peduncle length, single plant yield and 1000 seed weight. Traits such as plant height and days to fifty per cent flowering showed a left skewed distribution. The skewed distribution of a trait could be attributed to the presence of non-additive gene action and the trait is subjective to the influence of environmental variables. Considering kurtosis, number of basal tillers, days to fifty per cent flowering and single plant yield showed a leptokurtic distribution with a positive excess kurtosis, while all other traits exhibited a normal distribution. The traits with leptokurtic and platykurtic distribution are known to be controlled by fewer and large number of

genes, respectively. However the size of the germplasm and the variability accounted in the evaluated germplasm collection can influence the patterns of distribution.

Principal Component Analysis was applied as a reductionist approach of the multivariate data, to measure the importance and contribution of each component to total variance. PCA provides information on the independent impact of a particular trait to the total variance, wherein each coefficient of eigen vectors indicates the degree of contribution of every original variable, with which each principal component is associated. PCA analysis revealed that the first four components in the PCA analysis contributed to a maximum of 69.55 per cent of the variability among genotypes evaluated for different agro-morphological traits. These four principal components were retained based on the scree plot and threshold eigen value greater than 1 (Fig 2 and Table 3). The first principal component which accounted for 30.11 per cent variability is a measure of vegetative and inflorescence characters, most traits contributing in a positive direction. With an increase in plant height, the number of days to flowering, flag leaf length, flag leaf width, peduncle length, inflorescence length also tend to show an increasing trend. However, number of tillers and seed weight contributed in the negative direction on the first axis. This indicated that tall accessions tend to produce very low number of tillers. Upadhyay *et al.*, (2009) have reported such characteristic single stemmed tall accessions of foxtail millet from China. Similar to the present observations, PCA analysis based on morphometric traits in 26 foxtail millet accessions originating from China, Korea and Pakistan, revealed that most of quantitative characters such as panicle length, leaf number and internode number greatly contributed in positive direction, whereas characters such as tiller number, seed weight and panicle color contributed in negative direction on the first axis (Kim *et al.*, 2010).

In a study involving morphological characterization and PCA analysis of *Setaria viridis* and its close relatives, Layton and Kellogg (2014) also reported a similar finding that the first principal component axis explained 17 per cent of the variance, the second axis explained seven per cent, and the remaining axes contributed less than five per cent. The first principal component had high loadings for size of both floral and vegetative characters. The vectors for the vegetative characters loaded positively on both principal components, but primarily on the first axis.

The second component axis, accounting for 14.23 per cent of total variability is a measure of grain yield per plant which tends to increase with increasing flag leaf length and flag leaf width. This is anticipated since photosynthesis is the primary source of grain yield with flag leaf being the most essential organ for photosynthesis (Zhang *et al.*, 2015). At least 50% of photosynthetic products for grain are provided by the flag leaf (Li *et al.*, 1998). Thus the second component reflected the prominent role of flag leaf as a key photosynthetic organ towards increasing grain yield per plant. At the same time, higher plant yields are obtained with a reduction in the number of days taken to flowering.

The third component, accounting for 13.82 per cent of total variability is a measure of 1000 grain weight. Increased grain weight is found in plants producing more number of tillers, yet with comparatively lesser yield in Figure 3.

In cereals, the degree of panicle exertion is determined by peduncle elongation rate, and the panicle reaches its maximum length one day before heading (Ji *et al.*, 2005). The fourth component axis had high loadings for peduncle length accounting for 11.39 per cent of the total variability. An increase in peduncle length had a positive effect on seed yield and seed weight. Loadings of the fourth component indicated that with an increase in panicle length, there is a chance of good exertion during anthesis time, and hence aids in better pollination. This could result in better seed set, seed filling and is represented through a positive increase in seed yield and seed weight in foxtail millet, known for its drought hardiness. Similar findings have been also reported in upland rice grown under drought stressed environments (He and Serraj, 2012). Under drought stress, spikelet fertility in rice was found to be highly correlated with peduncle elongation. Also a strong correlation was observed between grain yield and spikelet fertility (He and Serraj, 2012). The scatter plot drawn between PC1 and PC2 depicted a clear pattern of grouping genotypes in the factor plane. Convex of the hull showing the outliers was occupied by the genotypes namely ISe278A, ISe144K, ISe143, GS2143, SiA2849, ISe1332, SiA2854 and Ise281. All the genotypes were widely scattered across different quarters (Figure 3). The prominent characters identified in a particular principal component as prime contributors to total variability have the tendency to hang together and can be used effectively for selection in crop breeding programmes.

For agronomic trait analysis, the quantitative traits are considered as features of productivity. These quantitative traits vary in their degree of expression and their inheritance is affected by gene interactions and the environment (Bhattacharjee *et al.*, 2007; Bjorklund *et al.*, 2009). Hierarchical cluster analysis based on quantitative traits divided foxtail millet genotypes into four main clusters and 10 sub-clusters (Fig. 4, Table 4). Cluster I comprised of 3 subclusters and included 14 accessions which were mostly characterized by tall plant architecture and with fewer tillers. Cluster II comprised of 15 accessions which were predominantly characterized by small grains having 1000 seed weight less than 3g. Among these accessions, the subclusters represented late flowering groups which took more than 53 days to attain fifty per cent flowering and intermediate flowering groups which took 48-53 days to attain fifty percent flowering. Cluster III comprised of 13 accessions, which were characterized by late flowering and high yielding genotypes. The grain yield per plant of most genotypes in this cluster was more than 20g. Group IV comprised of 13 accessions which were predominantly characterized by short stature, early flowering nature and with more number of tillers. The clustering results showed that among the nine quantitative traits, variation in the plant height, number of tillers, days to fifty per cent flowering, grain yield per plant, and 1000 seed weight had a major contribution in distinguishing the major clusters of foxtail millet at the morphological level. However, these clusters did not show any association with their geographical origin within India. This could be attributed to similarity in the pattern of phenotypic traits shared by genotypes dispersed over the various states.

Such a clustering pattern was also observed when a collection of foxtail millet germplasm comprising of 324 landraces which were developed independently and separated by geographical barriers, such as rivers, valleys and mountains were evaluated using 33 agronomic traits. Although the land races were well isolated and exhibited a wide array of phenotypic diversity, yet morphology based clustering showed out similar patterns, wherein great amount of the landraces in each cluster exhibited similar quantitative range for traits including the plant height, spike length, blade length of flag leaf, blade width of flag leaf, stem width, internode length, germination rate, plant survival at maturity, spike weight, grain weight, days to heading, growth periods, percent of threshing, grain number, harvest index, germination period, capacity, ratio of leaf



width/length, and flowering period (Lin *et al.*, 2012). Although geographic origins could be established across countries, based on morphological differentiation of 26 accessions of Foxtail Millet collected in Korea (15 accessions), China (7 accessions) and Pakistan (4 accessions), Kim *et al.* (2010) also observed that some Chinese accessions showed phenotypic similarity with Korean accessions, while some Chinese accessions showed similarity with Pakistan accessions.

The PCA analysis and dendrogram generated in the present study showed distinct clusters with important phenotypic attributes contributing towards diversity and provides a base for selection of desirable genotypes with specific traits for utilization in hybridization programmes.

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**Table 1.** Foxtail millet accessions and their source of origin

Genotype	Country/Province Source	Genotype	Country/Province Source
Ise1	Andhra Pradesh	ISE1054	Madhya Pradesh
Ise9	Andhra Pradesh	ISE1057	Meghalaya
Ise33Ak	West Bengal	ISE1204	USSR
Ise 40	Bihar	ISE1230	USSR
ISE43K	Bihar	ISE1332	Afghanistan
ISE54K	Bihar	ISE1858	Karnataka
ISE74A	Bihar	GS1918	Karnataka
ISE138	Kerala	GS2137	NBPGR
ISE143	Madhya Pradesh	GS2143	NBPGR
ISE144K	Madhya Pradesh	GS2184	NBPGR
ISE156	Madhya Pradesh	GS2227	NBPGR
ISE174	Tamil Nadu	SiA805	Andhra Pradesh
ISE204	Tamil Nadu	SiA147	Andhra Pradesh
ISE276B	Karnataka	SiA808	Andhra Pradesh
ISE278A	Karnataka	SiA2844	Andhra Pradesh
ISE278B	Karnataka	SiA2846	Andhra Pradesh
ISE281	Karnataka	SiA2847	Andhra Pradesh
ISE283	Karnataka	SiA2849	Andhra Pradesh
ISE317	Uttar Pradesh	SiA2854	Andhra Pradesh
ISE338K	Uttar Pradesh	SiA2855	Andhra Pradesh
ISE348K	Uttar Pradesh	SiA2859	Andhra Pradesh
ISE779	Gujarat	SiA2860	Andhra Pradesh
ISE789	Maharashtra	SiA3156	Andhra Pradesh
ISE792	Maharashtra	SiA326	Andhra Pradesh
ISE1047	Madhya Pradesh	Co(Te)7	Tamil Nadu
ISE1052	Maharashtra		



**Table 2.** Variation in quantitative traits of Foxtail millet

Variables	Mean	Min	Genotype	Max	Genotype	SD	CV (%)	Skewness	Excess Kurtosis
PH(cm)	142.40±1.86	103.96	ISe779	176.18	ISe138	13.25	9.31	-0.124	1.089
NBT	3.90±0.08	3.00	ISe338K	6.20	Ise1	0.57	14.60	1.507**	4.234**
DFF	52.27±0.53	40.00	ISe1332	60.00	ISe144K	3.77	7.21	-0.696*	1.417*
FLL(cm)	28.04±0.40	21.08	SiA2847	35.44	ISe144K	2.87	10.23	-0.234	0.210
FLW(cm)	2.04±0.04	1.24	ISe1332	2.46	ISe1204, SiA2860	0.25	12.12	-0.367	0.757
PL(cm)	23.69±0.46	16.24	Ise1	31.72	ISe144K	3.31	13.98	0.039	-0.029
IL(cm)	18.85±0.36	13.82	SiA805	23.56	ISe43K	2.54	13.49	-0.086	-0.934
SW(g)	2.92±0.04	2.33	ISe1057	3.63	ISe54K	0.30	10.18	0.061	-0.342
SPY(g)	20.49±0.65	13.00	ISe138	34.00	SiA2854	4.62	22.55	1.092**	1.408*

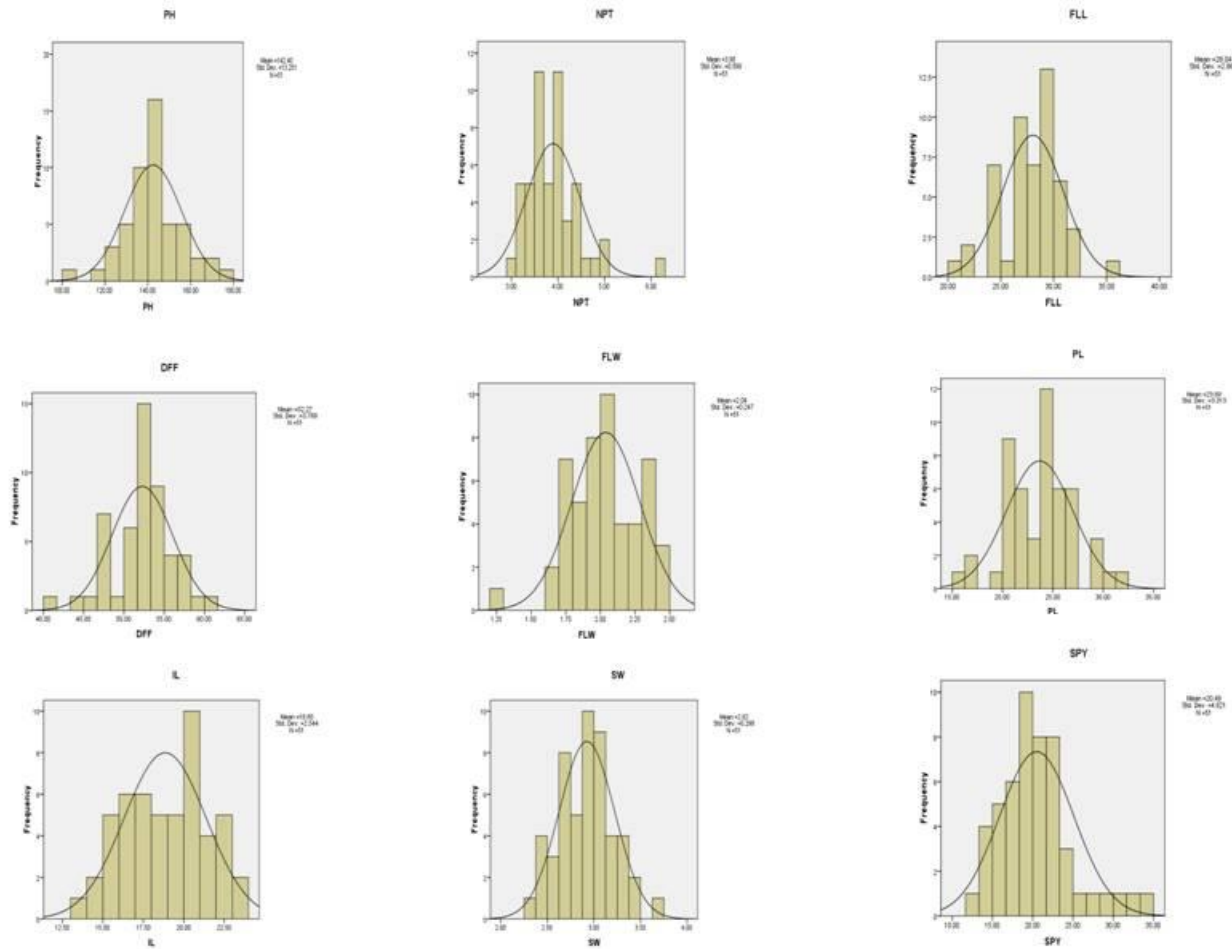




**Table 3.** Eigen value and percent of total variation and component matrix for the principal component axes

PC	PC 1	PC 2	PC 3	PC 4
Eigenvalue	2.71	1.28	1.24	1.02
% variance	30.11	14.23	13.82	11.39
Cumulative %	30.11	44.34	58.16	69.55
Component Matrix				
	PC 1	PC 2	PC 3	PC 4
PH(cm)	0.453	-0.278	0.313	-0.083
NPT	-0.170	0.062	0.616	-0.403
DFP	0.305	-0.426	-0.048	0.067
FLL(cm)	0.455	0.305	-0.009	-0.016
FLW(cm)	0.398	0.414	-0.127	-0.076
PL(cm)	0.267	-0.111	0.027	0.696
IL(cm)	0.469	0.087	0.223	-0.289
SW(g)	-0.100	-0.069	0.652	0.405
SPY(g)	-0.082	0.670	0.168	0.295

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**Figure 1a-9. Frequency distribution of different quantitative traits**

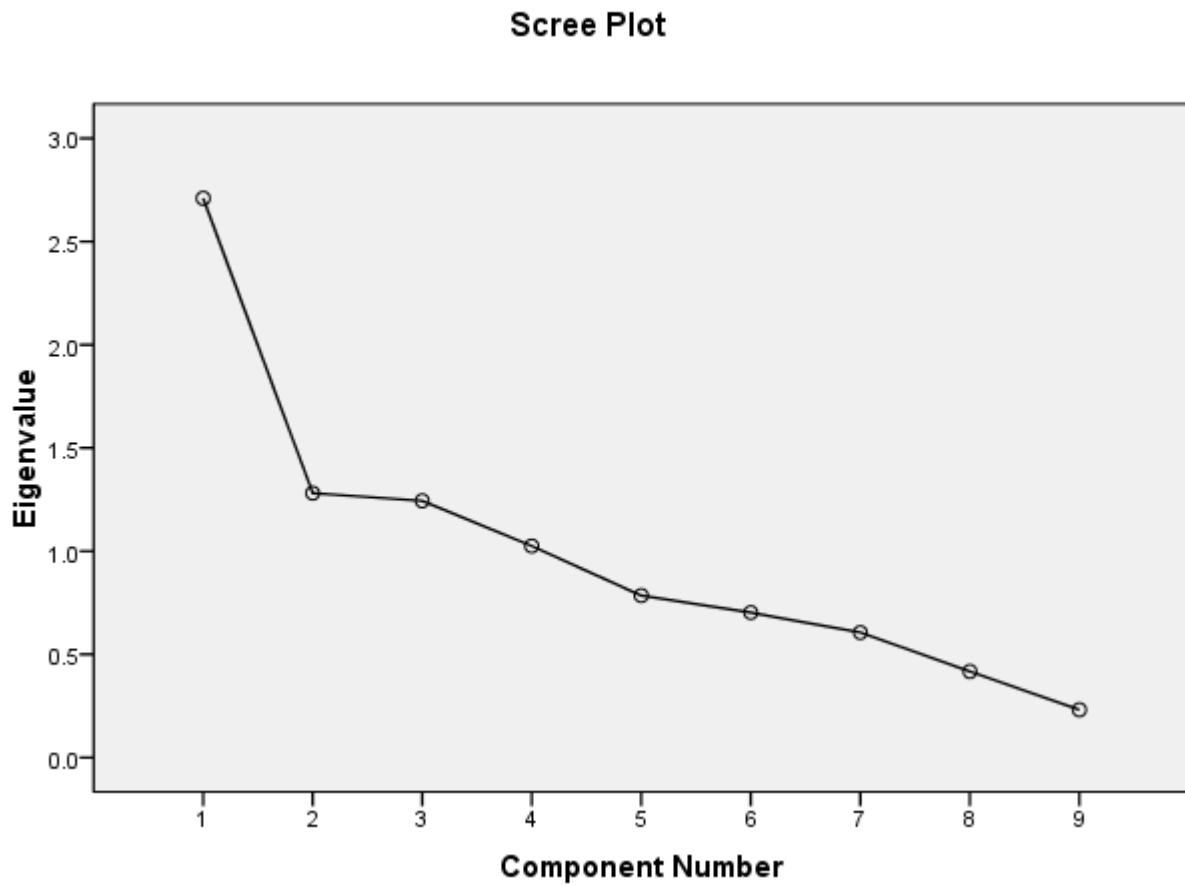


Figure 2. Scree plot showing the eigen value variation for nine quantitative traits in foxtail millet

### Component Plot

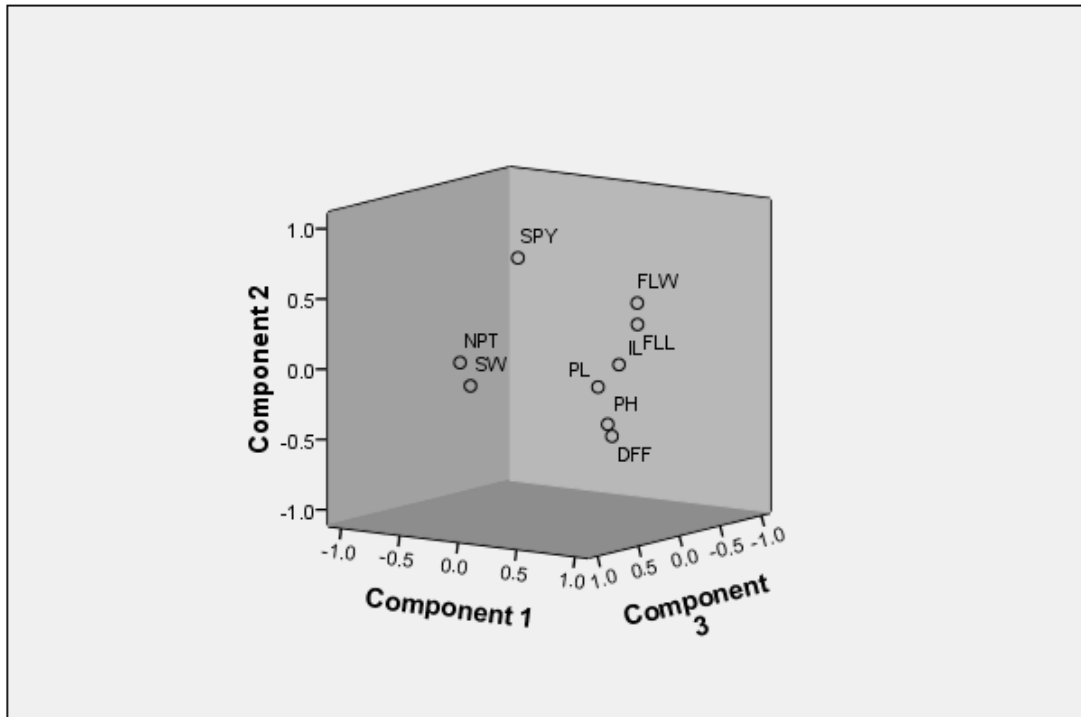


Figure 3: Component plots for the nine quantitative traits in foxtail millet germplasm

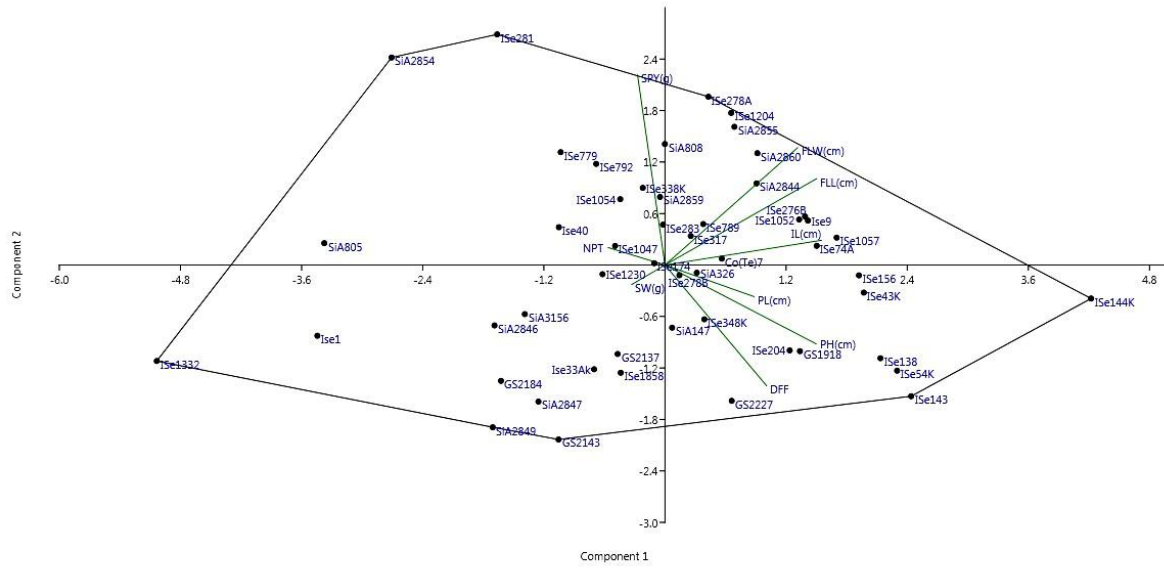


Figure 4: Distribution of Foxtail millet genotypes across the two components

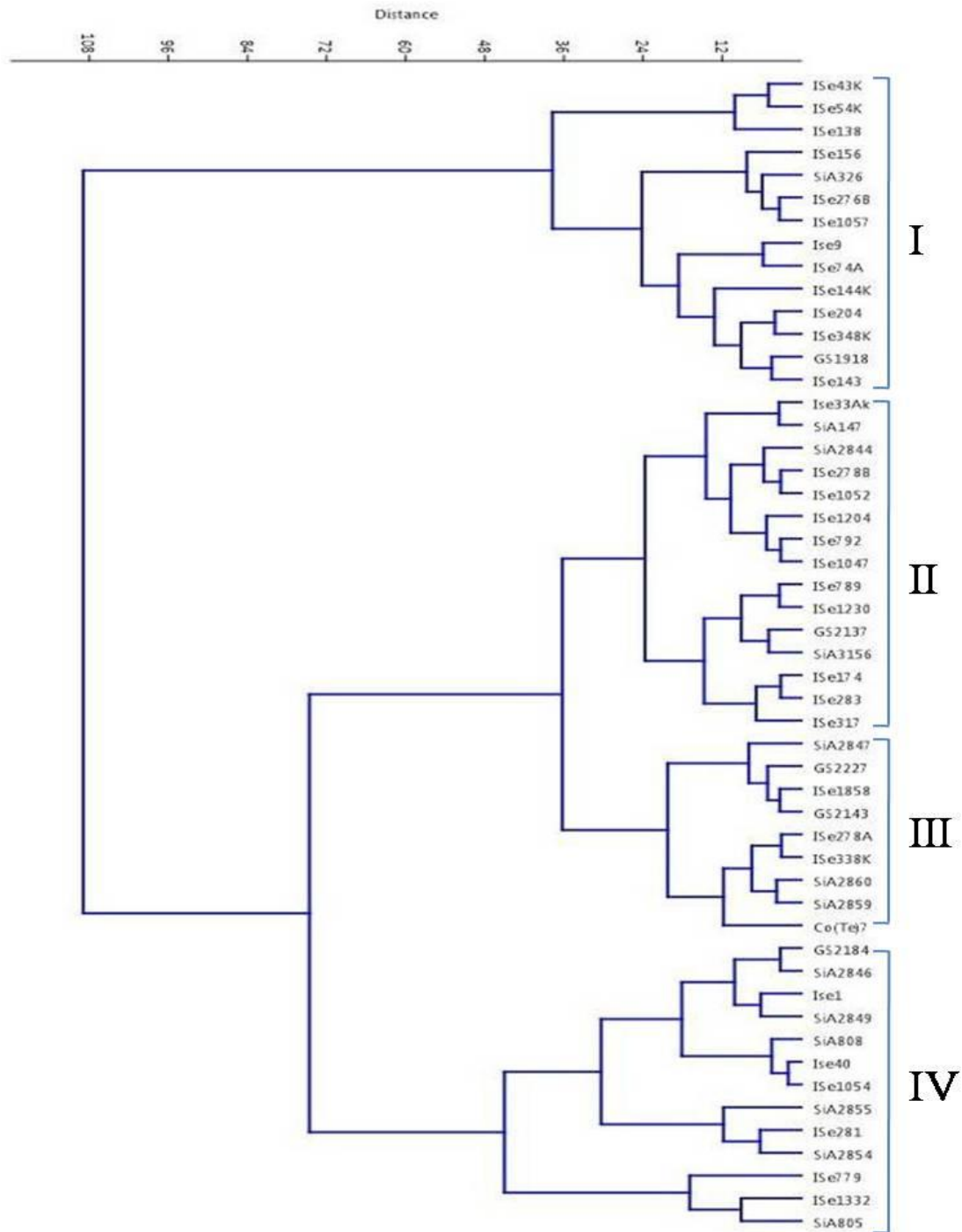


Figure 5: Dendrogram based on morphological traits in foxtail millet elite germplasm