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Unraveling Genetic diversity in Morphometric traits, Skewness Kurtosis, and Grain nutrients (Ca& Zn) composition through PCA and Clustering in core Germplasm accessions of Finger millet (*Eleusine Coracana* L).

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ABSTRACT

An investigation was conducted in the summer of 2022 at the College of Agriculture, Hasan, UAS, GKVK, Bangalore. The study aimed to uncover the genetic variability for yield, its component traits, and grain nutrient content in 97 core germplasm accessions of finger millet, along with three checks. The evaluation was performed using a Simple Lattice Design with two replications. Using k-means clustering the genotypes were categorized into fourteen clusters, of which cluster IX is the largest group, comprising 13 accessions followed by cluster V and XIII with 11 accessions, respectively indicating diversity among these clusters. Promising germplasm accessions identified in this study hold potential for utilization in future breeding programs aimed at enhancing existing cultivars. Analyzing the adjusted correlated and standardized two micronutrient contents of twenty-two germplasm and three checks using principal component analysis revealed the presence of two significant principal components (PCs) that collectively accounted for 94.48% of the observed variability. The skewness values for most of the quantitative traits studied in the germplasm were between -0.5 and 0.5. Skewness and kurtosis estimates are highly optimistic for Finger length(cm) (0.70) and ear head length(cm) (0.72) which means that more germplasms are below the mean than expected in a normal distribution.

Keywords- *Diversity, clustering, Grain nutrient, finger millet, Principal component analysis.*

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Introduction

Finger millet is a crop of antiquity and is known for its suitability for dry lands and tribal Agriculture. The resilience exhibited by this crop is helpful in their adjustment to different ecological situations and makes it an ideal crop for climate change and contingency planning.

It belongs to the family *Poaceae* (*Gramineae*) is an important food crop of India and it stands as the third most significant millet crop, following sorghum and pearl millet (Upadhaya *et al.*, 2007). It is a highly self-pollinated crop, allotetraploid (2n = 4× = 36) derived from the wild tetraploid progenitor *E. coracana ssp. Africana*. It is native to Ethiopian highlands.

Finger millet is typically called "Nutritious millet" because of its nutrition composition especially high carbohydrates 81.5%, dietary fibre content 18-20%, protein 6-13%, and minerals 2.7g/100g. The crop grains contain other important minerals like calcium 344mg/100g, phosphorus 283mg/100g, iron 3.9mg/100g, and zinc 2.79mg/100g (Chetan and Malleshi,2007; Shashi *et al.*, 2007). It has anti-diabetic, anti-diarrheal, antitumorigenic, antiulcer, anti-inflammatory, atherosclerogenic, antimicrobial, and antioxidantal properties (Devi *et al.*, 2014). It is the richest source of calcium content, providing 8–10 times more calcium than rice and wheat. Its excellent grain storage quality is attributed to polyphenol content (Chetan and Malleshi, 2007) which makes it an ideal crop for famine reserves. The crop residue is a source of fodder for livestock as its straw contains up to 61% digestible nutrients(anon.1996). Diabetic patients are motivated to consume finger millet and various small millets instead of rice because of no gluten content and low glycemic index (Chandrashekhar, 2010).

In Karnataka, finger millet is principally grown in Tumakuru, Hassan, Ramanagara, Kolar, Chikkaballapura, Mandya, Chitradurga, Bengaluru Rural, Chikkamagaluru, Mysuru, Bengaluru Urban, Chamarajanagar and Davanagere districts. Tumakuru district accounts for 22.7% and 18.6% of the area and production of finger millet followed by Hassan 11.3% and 10.7%, Ramanagara 10.4% and 14%, and Kolar 8.3% and 9.8% respectively (http://des.kar.nic.in). Bengaluru Urban district has the highest productivity of 3306 kg per hectare followed by Bengaluru Rural 2,702 kg/ha. As per the economic survey of 2019-20, the Government of Karnataka has launched the 'RAITHA SIRI' scheme to encourage millet farming.

Material and Methods

The material for the present study comprised 97 core germplasm accessions and 3 checks received from AICRP on small millets UAS, GKVK, Bangalore, and three checks *viz.*, KMR 204, ML 365, and GPU 48. The present study was carried out at the College of Agriculture Hassan UAS, GKVK, Bangalore. during summer in the year 2022, which is located at the Southern Transitional Zone (Zone 7) of Karnataka with an altitude of 827

m above Mean Sea Level (MSL) and at 33' N latitude and 75° 33' to 76° E38'. The materials used and the methodologies carried out for recording observations on yield and its attributed traits, nutrient analysis, and statistical techniques used for the analysis of recorded data in the present study.

Clustering of genotype using *k means* clustering.

The germplasm accessions were categorized using a model-based k-means clustering approach, by MacQueen in 1967.

$$J = \sum \sum \omega_{ik} \, \|X^2 - \mu_k\|^2$$

$i{=}k \; k{=}1$

$\|X2 - \mu k\|2$−Indicator of the distance between n data points from their respective cluster center
xi - Number of data points.
µk - Number of the cluster center

Coefficients of Skewness and Kurtosis:

Skewness, the third-order statistical measure, and kurtosis, the fourth-order statistical measure, were computed (Snedecor and Cochran, 1994) to ascertain the distribution characteristics of quantitative traits within the core accessions. Genetic expectations regarding skewness (-3/4 d2 h), as discussed by Fisher *et al.,* in 1932, reveal the nature of genetic control of the trait. The parameters 'd' represents additive gene effects, while 'h' represents dominant gene effects. Kurtosis provides insights into the proportion of genes influencing the traits. traits (Robson, 1956).

Grain nutrients

Grain nutrient content was carried out for the top twenty-two high-yielding accessions along with three checks. The detailed procedure for the estimation of nutrients is given below.

Calcium nutrient (mg/100 g)

The calcium content of the sample was estimated by preparing a mineral solution and titrating it against 0.01 N EDTA in the presence of an alkaline condition (AOAC, 1995).

Reagents:

- Standard EDTA solution (0.01N): Dissolve 1.861 gm of EDTA in 900 mL distilled water and make up the volume to 1000 ml.
- Std. Ca solution: Dissolve 0.6005 g portion of pure dried CaCO3 in 0.2 N HCl. The solution is boiled to expel the CO2 and dilute to 1 L.
- 10 % NaOH solution: Dissolve a 10 g portion of NaOH in about 90 mL distilled water and dilute to 100 ml.
- Murexide indicator: Mix 0.2 g of murexide with 40 g of powdered K2SO4.
- Buffer solution (pH 10): Add 142 mL of NH4OH to 17.5 g of NH4Cl and dilute to 250 ml with distilled water.
- Erichrome Black T indicator: Dissolve 0.2 g of the EBT powder 15 mL of triethanolamine and 5 ml of absolute ethanol.
-

a) Determination of calcium

Measure 5 ml of the digested sample onto a porcelain basin and dilute with 10 mL of distilled water.
Measure 5 ml of 10 % NaOH (pH of sample solution would reach more than 12).
About 0.5 g of murexide indicator is added.
Titrate the contents against std. EDTA with stirring until it becomes violet in color.

$$\text{Ca (mg/100 g)} = \frac{\text{T. V. Ca} \times \text{N of EDTA} \times 0.02 \times \text{total volume of digested sample}}{\text{Aliquot taken} \times \text{weight of the sample}}$$

Estimation of zinc

The zinc content of the sample was estimated using an Atomic Absorption Spectrophotometer and the results were expressed in ppm of the sample (AOAC, 1995).

Atomic Absorption Spectrophotometer (AAS)

Atomic absorption spectroscopy (AAS) uses the absorption of light to measure the concentration of gas-phase atoms. Since samples are usually liquids the analyte atoms or ions must be vaporized in a flame or graphite furnace. The atoms absorb ultraviolet or visible light and make transitions to higher electronic energy levels. The analyte concentration is determined by the intensity of absorption. Applying Beer-Lambert's law to AAS is appropriate due to variations in the atomization efficiency of the sample matrix and nonuniformity of concentration and path length of analyte atoms. The concentration is determined from a working curve after calibrating the instrument with standards of known concentration.

Procedure: Make suitable dilutions of tri/di acid extract and feed standard/sample to AAS having appropriate hollow cathode lamps. Record values and plot on graph paper.

$$\text{Zn (ppm)} = \frac{\text{Graph ppm} \times \text{volume made up} \times \text{dilution factor x } 1000}{1000 \text{ x weight of sample}}$$

Result and Discussion

Clustering of genotype using k-means clustering

In K-means, clustering tends to divide the 'n' objects into 'k' clusters in which each object within the cluster has the nearest mean. Each element in the data set is allocated to the cluster center with the minimum distance to that center, Kanavi *et al*., (2020).

Thus, based on the non-significance of Levene's test that resulted in the homogeneity of variances within the clusters the total genotypes were divided into eight clusters using non-hierarchical clustering. The mode of distribution of genotypes into eight clusters was random and it is based on all traits under consideration, not on the geographical distribution as mentioned by Kanavi *et al.,* (2020).

To investigate the significant differences between the clusters, a one-way analysis of variance was performed, for all relevant traits, the mean sum of squares between clusters was highly significant, indicating that genotypes within one cluster performed differently than genotypes in another trait (Table 1).

The estimated cluster means of fourteen clusters and the comparison of means are represented in Table 2. The distribution of cluster means along with range values with the cluster is represented in Fig 1. The maximum inter-cluster distance was observed between cluster III and cluster X (23.57), followed by cluster VIII and cluster X (23.45) indicating that the genotypes between these clusters are highly divergent. The minimum inter-cluster distance was observed between cluster V and cluster XI (12.19) and the genotypes within them are related to each other as shown in Table 3.

Clustering of the 100 accessions using k-means clustering resulted in fourteen clusters and the distribution of accessions into different clusters is represented in Table 4. Cluster IX had the most accessions 13, followed by Clusters VII and XIII with 11 accessions, cluster II and XII with 9 accessions, cluster V and XIV with 7 accessions, cluster I and IV with 6 accessions, cluster VI

with 5 accessions, cluster III with 4 accessions, cluster VIII and X with 3 accessions, indicating a high degree of heterogeneity among the accessions studied. Vaijayanthi (2013) used a similar clustering approach for clustering.

Table 1: Analysis of variance for yield and yield-related traits between clusters

Sl. No.	Source of variation	df	PH	TL	PL	DOM	FLL	FLW	EL	EW	FL	FW	GY	TW
1	Between cluster	13	254.68**	0.78**	68.3**	57.26**	55.91***	0.07***	8.50**	0.39**	7.73**	0.03**	111.25***	0.366***
2.	residual	86	41.29	0.105	6.75	10.62	15.38	0.008	0.72	0.063	0.73	0.005	9.022	0.112

*Significant @ P=0.05 level; **Significant@ P=0.01 level: *** Significant@ P=0.001.
PH= Plant height (cm) EL= Ear head length (cm)
TL= Tiller plant^{-1} EW= Ear head width (cm) FL=Finger length (cm)
PL= Peduncle length (cm) FW= Finger width (cm)
DOM= Days to maturity GY= Grain yield plant^{-1}(g)
FLL= Flag leaf length (cm) TW= Test weight (g)
FLW= Flag leaf width (cm)

Table 2: Cluster means of yield and yield-related traits based on k-means clustering.

Sl. No.	Cluster	PH	TL	PTL	FIL	FLW	PL	EL	EW	FL	FW	GY	TW	DOM
1	**I**	115.67	5.58	2.75	42.09	1.19	17.14	6.52	1.83	5.44	1.02	16.25	2.05	107.83
2	**II**	117.08	5.73	3.01	38.64	1.30	18.46	8.34	2.03	6.90	0.91	25.21	2.46	110.16
3	**III**	106.62	5.05	2.15	38.01	1.07	22.90	5.71	2.26	4.79	0.92	11.14	2.13	109.37
4	**IV**	114.43	4.50	3.00	37.02	1.39	18.93	8.08	2.25	7.01	0.88	22.92	2.48	107.16
5	**V**	103.85	5.06	2.68	37.30	1.34	24.41	6.67	2.58	5.75	0.94	16.87	2.38	104.35
6	**VI**	111.33	4.69	2.90	43.98	1.35	25.47	6.82	1.64	6.00	0.86	18.22	2.22	114.50
7	**VII**	113.09	5.18	2.99	35.39	1.27	18.37	5.89	2.33	4.97	0.99	22.78	2.28	109.27
8	**VIII**	111.18	4.70	3.29	41.06	1.14	19.01	6.55	1.94	5.46	0.87	27.07	2.10	105.50
9	**IX**	113.09	5.10	2.82	37.41	1.15	24.34	6.18	2.30	4.94	0.91	18.61	2.71	112.21
10	**X**	93.04	5.10	2.93	31.08	1.31	25.39	10.30	2.42	9.29	1.04	19.01	1.66	107.00
11	**XI**	96.31	5.18	2.86	35.67	1.33	24.65	7.42	1.84	6.06	0.87	20.36	2.36	106.90
12	**XII**	109.43	5.58	2.57	39.54	1.29	25.26	7.70	2.00	6.39	0.93	13.97	2.41	112.75
13	**XIII**	114.65	5.05	2.90	34.59	1.17	23.40	8.26	2.15	7.1	0.95	13.94	2.28	107.16
14	**XIV**	109.92	5.27	2.71	36.07	1.03	24.80	6.85	2.10	5.86	1.10	14.94	2.46	106.85

Table 3: Average intra and inter-cluster values between the 14 clusters from finger millet Core germplasm accessions

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
I	0	14.42018	15.97205	16.64217	14.97403	15.18292	12.78571	16.15288	14.26097	22.12682	15.59606	14.25046	14.21514	13.52876
II		0	21.13561	13.13885	16.84008	16.40237	13.57752	15.68601	15.60608	20.47767	14.70999	15.35719	13.21596	17.76072
III			0	20.58804	15.21278	18.49138	16.17285	19.29297	14.25144	23.5763	17.6857	15.89126	17.27243	14.71601
IV				0	14.49199	15.00385	13.2717	14.83637	15.34675	18.637	13.76549	16.99178	12.4562	17.9662
V					0	16.13663	13.07797	17.85851	12.96147	17.05871	12.19872	14.34808	13.13657	13.52106
VI						0	16.14572	16.8304	14.1611	22.40221	14.06297	14.3317	15.70145	17.55895
VII							0	14.18448	11.96794	19.46028	13.93109	16.18485	12.9209	14.85844
VIII								0	16.41028	23.45868	16.07555	20.74595	15.83377	17.81167
IX									0	20.59794	13.92561	13.89479	13.22241	13.08072
X										0	16.42477	19.42086	15.64349	19.3694
XI											0	14.08717	12.64606	14.86994
XII												0	14.1871	14.88367
XIII													0	13.16195
XIV														0

Table 4: Distribution of genotype in 14 clusters obtained using k-means clustering.

Sl. No.	Cluster	No. of genotypes	Genotype
1	**I**	6	GE57, GE70, GE74, GE75, GE130, GE220
2	**II**	9	GE69, GE71, GE72, GE73, GE80, GE137, GE142, GE145, GE303
3	**III**	4	GE61, GE63, GE152, GE232
4	**IV**	6	GE54, GE133, GE134, GE135, GE138, GE143
5	**V**	7	GE139, GE141, GE154, GE289, GE291, GPU48, ML365
6	**VI**	5	GE33, GE34, GE59, GE56, GE299
7	**VII**	11	GE51, GE58, GE67, GE68, GE76, GE78, GE79, GE140, GE147, GE153, GE217
8	**VIII**	3	GE53, GE131, GE132
9	**IX**	13	GE50, GE52, GE55, GE150, GE151, GE215, GE216, GE218, GE219, GE222, GE228, GE231, GE300

10	**X**	3	GE290, GE292, GE296
11	**XI**	6	GE59, GE234, GE235, GE293, GE294, GE298
12	**XII**	9	GE60, GE64, GE65, GE66, GE210, GE223, GE295, GE297, KMR204
13	**XIII**	11	GE136, GE144, GE146, GE148, GE149, GE212, GE213, GE214, GE230, GE233, GE301
14	**XIV**	7	GE62, GE224, GE225, GE226, GE227, GE229, GE288

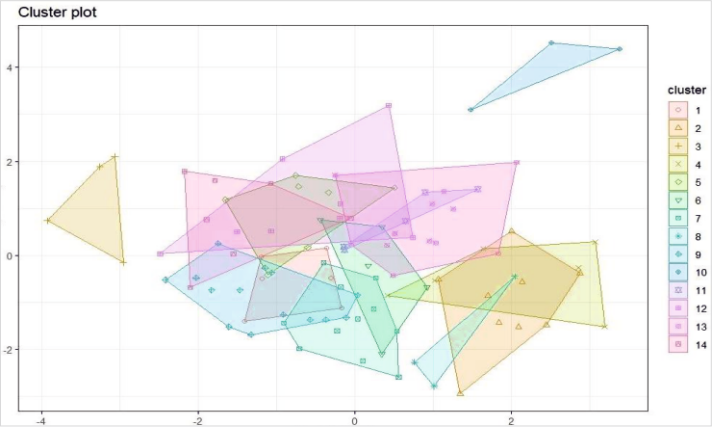

Fig 1. Cluster plot representing 14 clusters derived using finger millet germplasm for yield and yield attributing traits.

Furthermore, clusters VIII (27.07), II (25.21), IV (22.92), and VI (22.78) had higher grain yields, while clusters III, XIII, and XIV exhibited comparatively lower yields. As a result, genotypes from these clusters can be used in hybridization programs to improve yield (Manoj *et al*., (2022).

Grain nutrient analysis (Ca and Zn)

Principal component analysis (PCA) is a procedure for finding hypothetical variables (components) that account for as much of the variance in multidimensional data as possible. These new variables are linear combinations of the original variables. The technique of principal component analysis (Pearson, 1901) is described by Hotelling (1933). Calcium content ranged between 279 mg /100g to (GE78) to 365mg/100g (KMR204) and Zn content ranged between 21.56ppm (GE132) to 47.64ppm (GE 217) (Table 5).

Table 5: The calcium and zinc contents of twenty-five high-yielding finger millet core germplasm accessions.

Genotypes	Ca (mg/100 g)	Zn (ppm)
GE53	304.10	35.70
GE 143	309.80	33.80
GE 303	346.62	38.60
GE 68	322.00	39.20
GE 69	336.54	36.60
GE 131	326.00	28.80
GE 142	336.82	37.10
GE 79	304.20	30.00
GE 80	356.80	42.60
GE 71	302.40	34.52
GE 78	276.90	26.92
GE 133	298.20	26.12
GE 134	328.30	28.80
GE 59	298.20	30.96
GE 140	344.20	33.20
GE 230	298.40	37.60
GE 55	300.60	27.18
GE2 99	268.40	31.20
GE 56	328.20	40.20
GE 132	282.70	21.56
GE 137	278.20	29.20
GE 217	304.60	47.64
GPU48	320.00	29.58
ML365	320.00	26.76
KMR204	365.00	41.94

Correlation analysis

Grain yield is the result of the combined effect of several component characters and environment. Understanding the relationships between the characters is the most important criterion of association between the two metric characters. Using this genetic upgradation in one character can be brought up by the selection of another pair of characters. According to Grafius (1959), there may not be a gene for yield that operates solely through its components. As a result, understanding character association will aid in selecting traits to improve yield (Table 6).

Table 6: Phenotypic correlation of finger millet grain yield and its nutrient Traits

Traits	Grain yield plant^{-1}	Calcium (mg/100g)	Zinc (ppm)
Grain yield/plant	1.00	0.40	0.23
Calcium (mg/100g)	0.40	1.00	0.52*
Zinc (ppm)	0.23	0.52*	1.00

Genetic diversity (Principal Component Analysis)

The principle component analysis of adjusted correlated and standardized two micronutrient contents of twenty-two germplasm accessions and three checks resulted in two major PCs which together explained 94.48% of variability in Table 7. The 25 germplasms are scattered in all four quadrants of the dimensional graph Based on positioning, the 25 accessions could be grouped into 12 clusters (Fig 2). Of the 12 clusters, cluster XI with 4 accessions, clusters V and VII with 3 accessions, and the number of remaining clusters varied from 2 in clusters I, II, III, VI, VIII, and IX and 1 in clusters IV, VII, X, and XI.

Table 7: Number of principal components and Eigenvalues and contribution to total variability for grain nutrients contents in finger millet core germplasm accessions

PC	Eigenvalue	% Total Variance
1	644.71	94.48
2	27.86	4.082
3	9.80	1.43

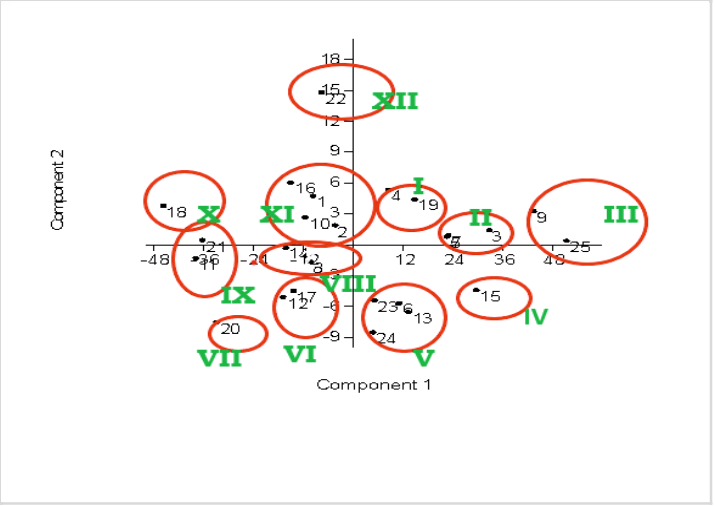

Fig:2 Grouping of genotypes into different clusters based on principal component analysis for grain nutrients in finger millet Core germplasm accessions

Skewness and kurtosis

The distribution study using skewness and kurtosis provides information about the nature of gene action (Fisher *et al.,* 1932) and the number of genes controlling the traits (Robson, 1956), respectively. Skewness and kurtosis are calculated using the frequency distribution of the characters mentioned (Kapur, 1981). β 1 = Skewness and β 2 = Kurtosis

If, β 1 > 0, then positively skewed, β 1 < 0, then negatively skewed, β 1 = 0,

then symmetric distribution for a normal distribution, skewness is equal to zero in the absence of gene interaction; it is greater and smaller than zero in the presence of average complementary and duplicate interactions, respectively If, β 2 > 1, then leptokurtic, β 2 < 1, then platykurtic

β 2 = 0, then mesokurtic.

The present study of skewness and kurtosis aimed at evaluating the 100-finger millet germplasm for 12 quantitative traits (Table 8). The estimate of PCV was higher than GCV for all characters and is due to the interaction of genotypes with the environment. The estimate of the phenotypic coefficient of variation (PCV) was high (>20%) whereas the genotypic coefficient of variation (GCV) was high (>20%) indicating high variability in the genotypes used for natural selection.

Table 8: Estimates of coefficients of skewness and kurtosis for yield and its components in finger millet germplasm

Traits	Kurtosis	Skewness
Plant height (cm)	0.72	1.03
Tillers plant^{-1}	-0.41	0.18
Flag leaf blade length (cm)	-0.41	0.02
Flag leaf blade width (cm)	0.64	0.43
Peduncle length (cm)	-0.61	-0.32
Finger length (cm)	0.41	0.70
Finger width (cm)	0.64	0.43
Ear head length (cm)	0.002	0.64
Ear head width (cm)	0.13	0.08
Days to maturity	1.7	-0.58
Grain yield plant^{-1} (g)	-0.73	0.24
1000 grain weight (g)	-0.43	0.32

The skewness values for most of the quantitative traits studied in the germplasm were between -0.5 and 0.5, except the trait "finger length," which had a value of 0.70, indicating a normal distribution in the population. Additionally, all the quantitative traits had kurtosis values greater than zero, indicating that the distribution of these traits had leptokurtic tails with a relatively peaked shape.

Skewness and kurtosis estimates are highly optimistic for Finger length (0.70) and ear head length (0.72) which means that more germplasms are below the mean than expected in a normal distribution. Distribution was negatively skewed for the traits, Days to maturity (-0.58), Plant height (-1.03), and Peduncle length (-0.32) which means that more germplasms are above the mean than expected in a normal distribution.

A high value of kurtosis was observed for the traits like days to maturity (1.7) followed by the Plant height (0.72) number showing leptokurtic distribution that indicates average complementary gene action (fig 3 to 14).

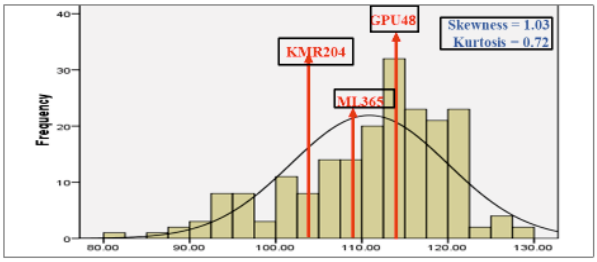

Fig 3:Platy kurtic and positively skewed distribution of Plant height in finger millet core germplasm accessions

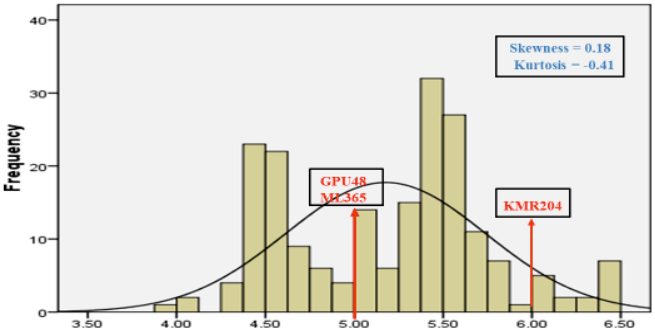

Fig 4: Platykurtic and Positively skewed distribution of tiller/plant in finger millet core germplasm accessions

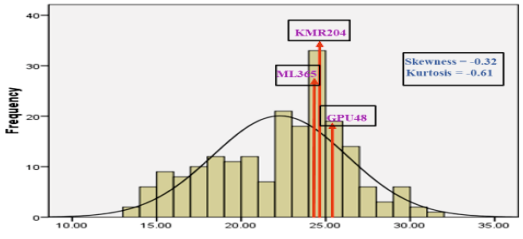

Fig 5:Platykurtic and Negatively skewed distribution of peduncle length in finger millet core germplasm accessions

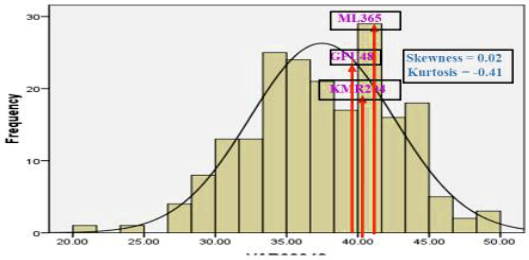

Fig 6:Platykurtic and Positively skewed distribution of Flag leaf length in finger millet core germplasm accessions

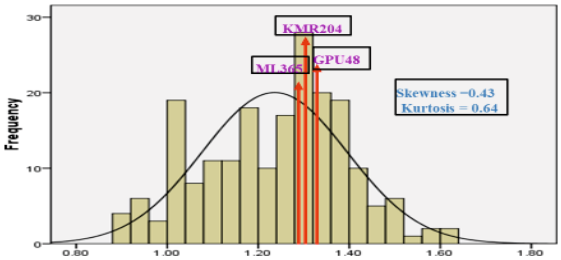

Fig 7:Platykurtic and Positively skewed of distribution of Flag leaf width in finger millet core germplasm accessions

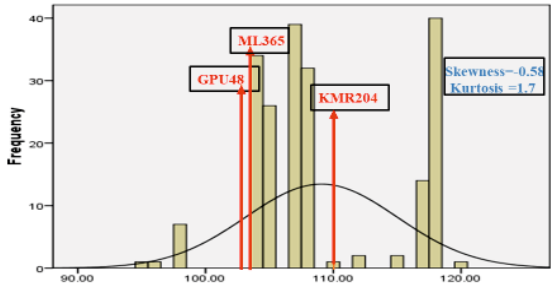

Fig 8: Platykurtic and Negatively skewed distribution of days to maturity in finger millet core germplasm accessions

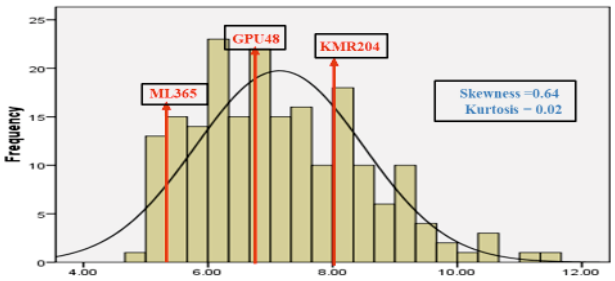

Fig 9 : Platykurtic and Positively skewed distribution of Ear head length in finger millet core germplasm accessions

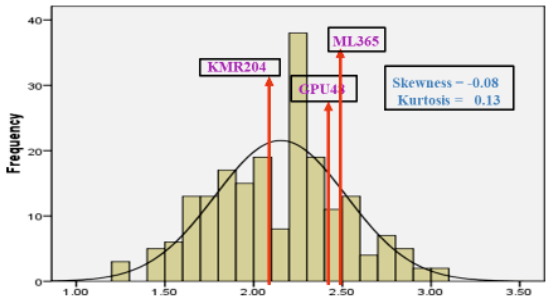

Fig 10: Platy kurtic and Negatively skewed distribution of ear head width in finger millet core germplasm accessions

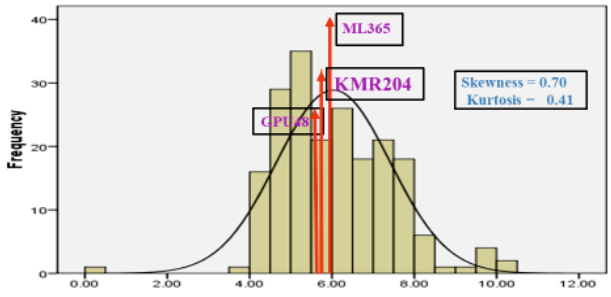

Fig 11: Platykurtic and Positively skewed distribution of finger length in finger millet core germplasm accessions

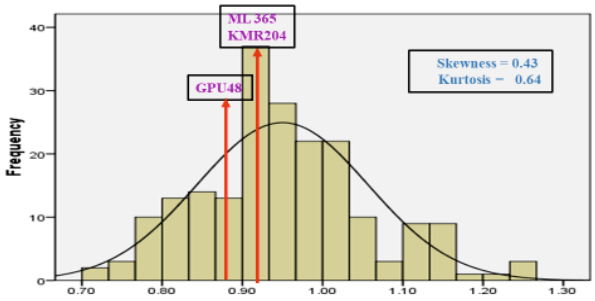

Fig 12: Platykurtic and Positively skewed distribution of finger width in finger millet core germplasm accessions

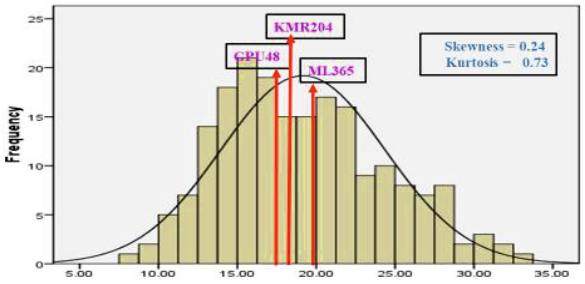

Fig 13: Platykurtic and Positively skewed distribution of Grain yield/plant in finger millet core germplasm accessions

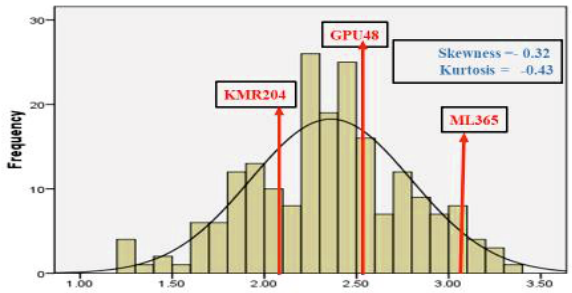

Fig 14: Platykurtic and Negatively skewed distribution of Test weight in finger millet core germplasm accessions

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