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Characterization of Some ex situ Conserved Finger Millet (*Eleusine coracana* (L.)) Germplasm Accessions in Sri Lanka

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ABSTRACT

Finger millet (Eleusine coracana (L.) Gaertn.) is a highly nutritious and important food crop widely cultivated in the arid and semiarid regions in the world. Therefore it is worthy to be subjected to crop improvement programs. Germplasm collection and characterization are preliminary and important steps in crop improvement programmes. This study was conducted to characterize randomly selected 24 finger millet germplasm accessions conserved at the plant Genetic Resource Centre, Gannoruwa, Sri Lanka using 14 quantitative characters. The maximum positive and significant coefficient of variation was observed between weight of grain per ear and weight of sun dried ear. Phenotypic correlation between weight of grain per ear was highly significant and positively associated with days to flowering, flag leaf width, flag leaf length, plant height, culm thickness, finger length, finger width, days to maturity and weight of sun dried ears, flag leaf width, flag leaf length, plant height, culm thickness, finger length, finger width, days to maturity and weight of sun dried ears. The principal component analysis revealed that the first five component with Eigen values greater than 0.87 contributed about 85.5% of total variability. The twenty for finger millet accessions grouped in to four main clusters in the cluster analysis. Results of cluster analysis could be used in the crop breeding and conservation programmes.

KEYWORDS: *Finger millet, germplasm accessions, genetic diversity, principal component analysis, quantitative characters*

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1. INTRODUCTION

Finger millet (*Eleusine coracana* (L.) Gaertn.) is an allotetraploid (2n=4x=36) of the family Poaceae and subfamily, Chloridoideae and is commonly known as "Kurakkan" or "Kurahan" in Sri Lanka. It is an important food crop widely cultivated in the arid and semiarid regions of the world. It is native to Ethiopia and was introduced to India about 3000 years ago. Finger millet has been cultivated in Sri Lanka since ancient times and considered as the second staple food after rice in most of the rural areas. Finger millet is a highly nutritious crop with the highest amount of Calcium and Potassium content out of all cereal and millets. It contains a high amount of dietary fiber and have the ability of lowering blood glucose and cholesterol levels. Recently increasing attention is paid to improve finger millet due to above qualities and its inherent capacity to tolerate several abiotic stresses including water deficit and its adaptability to marginal soils with low fertility (Gana *et al*., 2013).

Germplasm is the basic raw material for any crop improvement programme. Characterization of germplasm accessions to identify true genetic diversity is also essential to use in crop improvement. About 200 finger millet germplasm accessions are being conserved at the seed gene bank of the Plant Genetic Resource Centre (PGRC), Gannoruwa, Sri Lanka. Some of those germplam accessions have been characterized by Kannagara *et al*., (2011) and Wakista *et al*., (2015a, 2015b, 2015c and 2015d) using microsatellite markers and by Senanayaka *et al*., (2008) using AFLP markers. But largely those accessions are uncharacterized.

Multivariate statistical techniques are widely used in analysis of genetic diversity irrespective of whether it is morphological, biochemical or molecular marker-based. Among the multivariate techniques Principal Component analysis (PCA) and cluster analysis are very useful in germplasm characterization. Multivariate analysis has been used frequently in genetic diversity analysis many crops such as finger millet (Dagnachew *et al*., 2012, Ulaganathan & Nirmalakumari, 2015) and rice (Gana *et al*., 2013).

This study was undertaken to characterize some randomly selected conserved finger millet germplasm accessions using morphological markers to reveal their genetic relatedness.

2. MATERIALS & METHODS

2.1. Plant Material

Randomly selected 24 finger millet germplasm accessions obtained from the ex situ conserved accessions at the seed gene bank of Plant Genetic resource Centre (PGRC), Gannoruwa, Sri Lanka were used in this study (Table 1). These 24 accessions comprised of 19 local accessions collected from different geographical locations, two (02) Indian accessions, two (02) Zimbabwean accessions and two recommended Sri Lankan varieties. Seeds of the germplasm accessions were obtained from PGRC Gannoruwa.

Table 1. Details of the 24 finger millet germplasm accessions used for the study

Accession No.	Accession Name	Abbreviation for the accession	Origin of accession	Organization
000122	Kobey Kurakkan	KOKU (MONERA)	Moneragala	PGRC
000258	Kurakkan	KU (HAMBA)-1	Hambanthota	PGRC
000923	RAG15-6	INDIA-1	India	Unknown
000959	Kurakkan	KU (N.ELIYA)	NuwaraEliya	PGRC
000963	CO-10	INDIA-2	India	RARS/ AK
000967	Line 40	L40 (HAMBA)	Hambantota	RARS/ AK
001233	Kalugal Kurakkan	KLKU (MATALE)	Matale	PGRC
001815	Bala Kurakkan	BAKU (KANDY)	Kandy	PGRC
003011	Kurakkan	KU (HAMBA)-2	Hambanthota	PGRC

003021	Maha Mora Kurakkan	MMKU (HAMBA)	Hambantota	PGRC
004989	Kurakkan	KU (PUTTA)	Puttalam	PGRC
006580	Kurakkan	KU (MONERA)	Moneragala	PGRC
006586	Kurakkan	KU (MATALE)	Matale	PGRC
007089	Kurakkan	KU (KURUNE)	Kurunegala	PGRC
007109	SDFM 1143	ZIMBABWE -1	Zimbabwe	ICRISAT
007123	SDFM 2564	ZIMBABWE - 2	Zimbabwe	ICRISAT
007769	Kurahan	KU (POLON)	Plonnaruwa	PGRC
007777	Kurakkan	KU (BADULLA)	Badulla	PGRC
008389	Haramus Kurakkan	HAKU (ANU)	Anuradhapra	PGRC
008470	Makala Kurakkan	MAKU (MONERA)	Moneragala	PGRC
008796	Kurakkan	KU (KEGALLE)	Kegalle	PGRC
009083	Kaha Kurakkan	KAKU (N.ELIYA)	NuwaraEliya	PGRC
09294	Ravi	RAVI	Recommended variety	PGRC
10326	Ravana	RAVANA	Recommended variety	PGRC

2.2. Morphological Characterization

2.2.1. Experimental Design

Seeds of each accession were sown in small plastic pots filled with a mixture of soil and compost (3:1), in the green house of the Department of Botany of University of Sri Jayewardenepura. About 20 days old seedlings were transplanted separately in to large pots (22 cm diameter) filled with the same mixture of soil and compost as each pot contained three (03) plants. All accessions were arranged in a Randomized Complete Block Design (RCBD) with 12 individuals from each accession.

2.2.2. Data Collection

Fourteen quantitative characters [days to flowering, flag leaf length (mm), flag leaf width (mm), plant height (cm), clum thickness (mm), finger number, finger length (mm), finger width (mm), clum branching, days to maturity, weight of sun dried ear (g), weight of grain per ear (g), 1000 grain weight (g) and number of productive tillers] were recorded at different growth stages of the crop. Characters were scored following International Plant Genetic Resource Institute (IPGRI) descriptors developed for finger millet.

2.3. Statistical Analysis

2.3.1. Descriptive statistics

Descriptive statistics mean, minimum, maximum and standard deviation for each character were obtained using MINITAB17 software.

2.3.2. Principal Component Analysis

Principal Component Analysis for 17 quantitative characters was performed using MINITAB14 software. As suggested by Johnson & Wichern (1988) principal components with Eigen values greater than one was considered.

2.3.3. Cluster analysis

Hierarchical clustering of complete linkage method with squared Euclidian distance was performed using MINITAB17 software. Data of all quantitative characters were standardized to a mean of zero and a variance of one before clustering to avoid bias that arise due to differences in measurement scales.

3. RESULTS & DISCUSSION

Twenty two finger millet germplasm accessions and two recommended finger millet varieties were characterized using 14 quantitative characters. Out of 14 quantitative characters one character (number of productive tillers) was monomorphic across all 24 accessions and other 13 characters were polymorphic. Wide range of genetic variability was observed for different characters and their mean, minimum and maximum values are given in Table 2.

Earliness is an important agronomic trait considered while breeding for high yielding varieties. The number of days to flowering ranged from 66.92 to 94.08 with an average value of 81.26. Germplasm accession Co-10

(collected from India) had lowest and Makala Kurakkan collected from Moneragala had the highest value for days to flowering. Plant height and culm thickness also are important traits as those affect on the resistance to lodging.

Accession KU (Monera) – collected from Moneragala showed shortest plant and accession Haramus Kurakkan collected from Anuradhapura showed plant highest.

Table 2. Genetic variability for 14 quantitative characters in 24 finger millet accessions

Character	Mean	Minimum	Maximum	SD
Days to flowering	81.26	66.92	94.08	6.57
Flag leaf length (mm)	280.82	146.00	346.25	49.55
Flag leaf width (mm)	8.44	6.00	12.00	1.02
Plant height (cm)	82.44	58.13	101.53	9.92
Culm thickness (mm)	21.03	16.64	26.25	2.15
Fingernumber	6.44	4.33	8.08	1.17
Finger length (mm)	55.64	43.25	91.58	8.95
Fingerwidth (mm)	9.37	7.92	10.33	0.51
Culm branching	1.66	0.42	2.92	0.72
Productive tillers	0.00	0.00	0.00	0.00
Daystomaturity	125.19	96.00	146.00	13.35
weight of sun dried ear (g)	3.05	1.11	4.60	0.90
Weight of grain per ear (g)	2.45	0.94	3.82	0.77
1000 grain weight (g)	2.19	1.76	2.64	0.22

SD – Standard Deviation

Knowledge on the association between yield and other biometrical traits provide ample opportunities for improvement of crop. The correlation between characters may exist due to various reasons. The association of all quantitative characters was estimated by correlation analysis (Table 3). Out of all studied quantitative characters, days to flowering (days to flowering (0.485), flag leaf width (0.627), flag leaf length (0.710), plant height (0.636), culm thickness (0.408), finger length (0.505), finger width (0.406), days to maturity (0.723) and weight of sun dried ears (0.982) had significant and positive correlation with weight of grain per ear at ≤0.005 level. Weight of grain per ear shows positive correlation with the highest number of characters indicated that all those characters including days to flowering, flag leaf width, flag leaf length, plant height, culm thickness, finger length, finger width, days to maturity and weight of sun dried ears could be simultaneously improved and it also suggested that increasing any one of them would lead to improvement of the other character. Significant and positive association of grain yield per plant with days to flowering, productive tillers per plant, plant height, 1000 grain weight, flag leaf sheath length, days to maturity, flag leaf blade length and finger width have been reported (Ulaganathan & Narmalakumari., 2015), with days to flowering, flag leaf sheath length, flag leaf blade length and 1000-grain weight (Kadam *et al*., 2009) and productive tillers per plant (Dagnachew *et al*., 2012).

Grain yield per plant was equal to the weight of grain per ear with respect to all accessions used in this study as there were no productive tillers. Significant negative correlation (at ≤ 0.005 level) observed for days to flowering with clum branching (-0.436) indicated increasing one character would lead to decrease in another character. The character 1000-grain weight did not show any significant correlation with any of other characters.

Table 3. Phenotypic correlation coefficient for 13 quantitative characters in 24 finger millet accessions

	DF	FLW	FLL	PH	CT	FN	FL	FW	CB	DM	WSDE	WGE	1000 GW
DF	1.000												
FLW	0.205	1.000											
FLL	0.414*	0.721*	1.000										
PH	0.074	0.425*	0.580*	1.000									
CT	0.031	0.451*	0.635*	0.665*	1.000								
FN	-0.106	0.489*	0.608*	0.321	0.668*	1.000							
FL	0.206	0.530*	0.399	0.414*	0.281	-0.144	1.000						
FW	0.256	0.247	0.340	0.343	0.186	-0.119	0.278	1.000					
CB	-0.436*	0.075	0.187	0.235	0.309	0.353	0.014	0.315	1.000				
DM	0.440*	0.351	0.461*	0.682*	0.414*	-0.073	0.622*	0.384	0.018	1.000			
WSDE	0.457*	0.674*	0.722*	0.634*	0.469*	0.263	0.517*	0.411*	-0.025	0.718*	1.000		
WGE	0.485*	0.627*	0.710*	0.636*	0.408*	0.203	0.505*	0.406*	-0.054	0.723*	0.982*	1.000	
1000GW	0.262	0.006	-0.048	0.048	-0.082	-0.014	-0.065	-0.155	-0.140	0.365	0.134	0.136	1.000

*Significant at $P \leq 0.05$ level

DF - Days to flowering , FLW - Flag leaf width(mm), FLL - Flag leaf length(mm), PH- Plant height (cm) , CT - Culm thickness(mm), FN - Finger number, FLM - finger length(mm), FW - Finger width (mm), CB - Culm branching, DM - Days to maturity, WSD - Weight of sun dried ear(g), WGE - Weight of grain per ear(g), 1000GW - 1000 grain weight (g)

Table 4. Principal Components showing the Eigen values, proportion of variation and total proportion of variation across axis

Principal component	Eigen value	Variation (%)	Total variation explained across axis (%)
1	5.58	42.9	42.9
2	2.21	17.0	59.9
3	1.38	10.6	70.5
4	1.07	8.3	78.8
5	0.87	6.7	85.5
6	0.65	5.0	90.5
7	0.47	3.6	94.1
8	0.30	2.3	96.5
9	0.21	1.6	98.1
10	0.10	0.8	98.9
11	0.08	0.6	99.5
12	0.05	0.4	99.9
13	0.01	0.1	100.0

Table 5. Principal Component analysis for 13 quantitative traits in 24 finger millet germplasm accessions – non-rotated loadings

Character	**PC1**	**PC2**	**PC3**	**PC4**	**PC5**
Days to flowering	0.181	0.423	-0.227	0.220	0.413
Flag leaf width (mm)	0.318	-0.103	-0.140	0.295	-0.186
Flag leaf length (mm)	0.363	-0.148	-0.155	0.219	0.161
Plant height (cm)	0.329	-0.115	0.117	-0.305	-0.138
Culm thickness (mm)	0.286	-0.341	-0.091	-0.122	-0.082
Finger number	0.168	-0.494	-0.435	0.032	0.114
Finger length (mm)	0.261	0.162	0.309	0.105	-0.570
Finger width (mm)	0.201	0.051	0.536	0.074	0.566
Culm branching	0.060	-0.456	0.364	-0.320	0.238
Days to maturity	0.327	0.257	0.130	-0.378	-0.107
Weight of sun dried ear (g)	0.388	0.122	-0.050	0.059	0.028
Weight of grain per ear (g)	0.380	0.160	-0.038	0.058	0.043
1000 grain weight (g)	0.041	0.268	-0.398	-0.663	0.118

Principal Component Analysis (PCA) was performed for 13 polymorphic quantitative characters. The principal component analysis (PCA) can be used to identify the plant traits which contribute the most for the observed variation among the studied finger millet germplasm accessions. The PCA results revealed that the first five components with Eigen value of greater than 0.87 contribute about 85.5% of total variability in 24 finger millet germplasm accessions involving all the 13 quantitative characters studied (Table 4). According to Johnson & Withern (1988) principal component with Eigen values greater than one should be considered. But fifth PC with Eigen value 0.87 was also considered as it also was a considerable value. A variance of42.9%, 17.0%, 10.6%, 8.3% and 6.7% were extracted from the first to the fifth component respectively.

Table 5 shows the corresponding eigen values which gives an idea about the importance of traits towards the principal components. First principal component accounted for 42.9% of the total variation in the population. As shown in Table 5, Flag leaf width (0.318), Flag leaf length (0.363), Plant height (0.329), Days to maturity (0.327), Weight of sun dried ear (0.388) and Weight of grain per ear (0.380)contributed more to the variation in PC1 indicating their significant importance for the first principal component. All traits contributed positive to the first component. Second principal component contributed 59.9% of total variation. Characters that contributed to the second component include Days to flowering (0.423), culm thickness (-0.341), finger number (-0.494) and culm branching (-0.456). The third principal component contributed for 70.5% of the total variation.

The variation in the third principal component was mainly due to finger number (-0.435), finger length (0.309), finger width (0.536), culm branching (0.364) and 1000 grain weight (-0.398). Likewise, Plant height (-0.305), Culm branching (-0.320), Days to maturity (-0.378) and 1000 grain weight (-0.663) were the major contributor to the variation in the fourth principal component. Fourth principal component cumulatively accounted for 78.8% of the total variation in the population. The fifth principal component cumulatively contributed 85.5% of the total variation. The variability in the fifth component was attributed mainly due to Days to flowering (0.413) finger length (-0.570) and finger width (0.566).

Data from first two components were used to derive PCA score plot and loading plot (Figure 1 and 2). The studied 24 accessions were divided into four groups (A, B, C and D) based on the first two principal components as shown in PCA score plot (Figure 1). Group A comprised of two local accessions [KU(MONERA) - Kurakkan collected from Moneragala, KAKU(N.ELIYA) – KahaKurakkan collected from NuwaraEliya], one exotic accessions (ZIMBABWE-2) and two recommended varieties (RAVI and RAVANA) while five local accessions [BAKU(KANDY) – BalaKurakkan collected from Kandy, KU(HAMBA)-2 – Kurakkan collected from Hambanthota, KU(MATALE) – Kurakkan collected from Matale, L40(HAMBA) – line 40 collected from Hambanthotaand MAKU(MONERA) – Makala Kurakkan collected from Moneragala were in group B. Group C comprised of five local accessions [KU(POLON) – Kurakkan collected from Polonnaruwa, KU(N.ELIYA)-Kurakkan collected from NuwaraEliya, KOKU(MONERA)- KobeyKurakkan collected from Moneragala, KU(BADULLA)-Kurakkan collected from Badulla and KU(PUTTA) – Kurakkan collected from Puttalum] one exotic accession (INDIA-2). Local accessions; KU(HAMBA)-1- Kurakkan collected from Hambanthota, KU(KURUNE)-Kurakkan collected from Kurunegala, KU(KEGALLE) – Kurkkan collected from Kegalle, MMKU (HAMBA) – Maha Mora Kurakkan collected from Hambanthota and one Indian accessions (INDIA-1) were in group D (Figure 1).

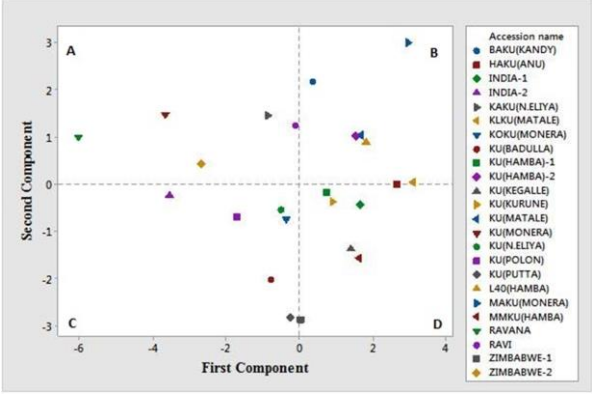

Figure 1. Score plot showing the grouping of studied finger millet germplasm accessions

Similarly, the recorded characters were divided into two groups (X and Y) depending on the first two principal components as shown in PCA loading plot (Figure 2).

The group X consists of Days to flowering, days to maturity, Finger length, Finger width, weight of sun dried ear, weight of grain per ear and 1000 grain weight whereas Plant height, Flag leaf length, flag leaf width, culm thickness, culm branching and finger number had been grouped in the group Y.

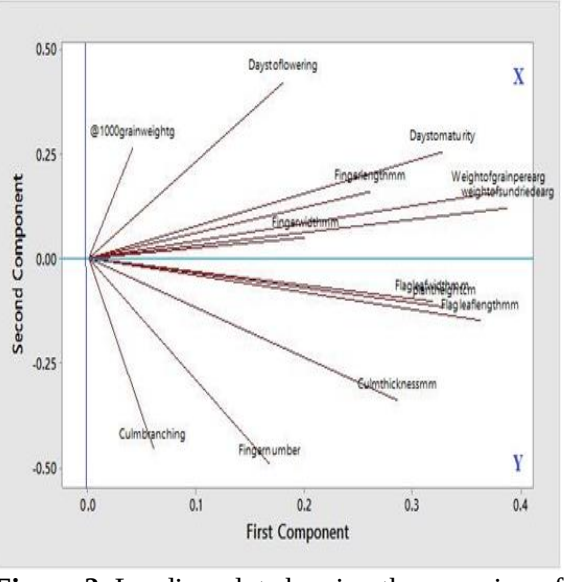

Figure 2. Loading plot showing the grouping of the 13 quantitative characters

Dendrogram constructed based on squared Euclidian distance comprised of four (04) sub clusters as Cluster I, Cluster II,Cluster III and Cluster IV (figure 3). Cluster I comprised of one local accession collected from Moneragala(KU(MONERA)) and one accession collected from India (INDIA-2) and one local recommended variety (RAVANA).

A local accession collected from Moneragalanamed as Makala Kurakkan (MAKU(MONERA) separated from remaining clusters at the similarity level of about 30 showing their genetic dissimilarity with the accessions of four clusters. Cluster II consisted

of five accessions and three of them were local accessions [KU(POLON), KAKU(N.ELIYA) and BAKU(KANDY)] collected from Plonnaruwa, NuwaraEliya and Kandy.

A Zimbabwian accession (ZIMBABWE-2) and other recommended variety (RAVI) were other two constituents of that cluster. Kurakkan accession collected from Polonanaruwa (KU(POLON)) has groped together with Zimbabwian accession (ZIMBABWE-2) within a sub group of that cluster showing their genetic relatedness even when they represent different geographical origins. Eight accessions were within cluster III and seven of them were local accessions [HAKU(ANU)-Haramuskurakkan collected from Anuradhapura, KU(HAMBA)-2-Kurakkan collected from Hambanthota, KLKU(MATALE)-KalugalKurakkan collected from (Matale), KU(MATALE)-Kurakkan collected from Matale, L40(HAMBA)-Line 40 collected from Hambanthota, KU(KURUNE)-Kurakkan collected from Kurunegala, KU(HAMBA)-1-Kurakkan collected from Hambanthota] and only one (INDIA-1) was with exotic origin.

Though three accessions collected from same districtsHambanthota [KU(HAMBA)-2, L40(HAMBA) and KU(HAMBA)-1] could be seen within this cluster those were sub grouped with other three accessions collected from three different districts Anuradhapura, Matale and Kurunegala respectively.

This observation proves that genetic relatedness is irrespective to their geographic origin. Cluster IV comprised of six local accessions [KU(PUTTA)-Kurakkan collected from Puttalum, KU(KEGALLE)-Kurakkan collected from Kegalle, MMKU(HAMB)-Maha Mora Kurakkan collected from Hambanthota, KU(BADULLA)-Kurakkan collected from Badulla, KU (N.ELIYA)-Kurakkan collected from NuwaraEliya, KOKU(MONERA)-KobeyKurakkan collected from Moneragala] and one accession collected from Zimbabwe (ZIMBABWE-1) (Figure 3).

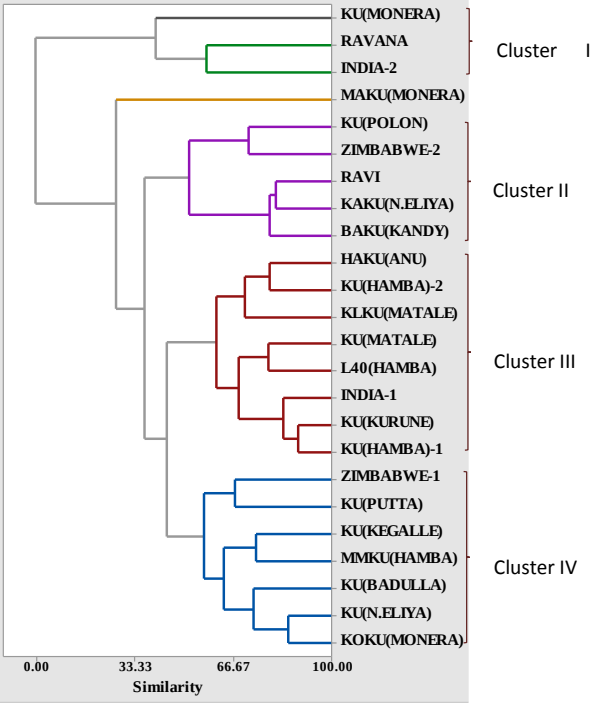

Figure 3. Dedrogram of finger millet germplasm accessions constructed based on squared Euclidian distance

This clustering pattern could be used in choosing diverse genotypes to use in breeding and conservation programmes. The crossing among the genetically distant accessions would produce individuals with high heterosis. The accession collected from Moneragala (MAKU MONERA) formed solitary cluster and found to be more variable.

4. CONCLUSIONS

Characterization of available germplasm accessions is very important in crop improvement programmes and morphological markers play an important role in germplasm characterization. Fourteen quantitative characters were used in the study and Principal Component Analysis revealed that days to flowering, flag leaf width, flag leaf length, plant height, culm thickness, finger length, finger width, days to maturity and weight of sun dried ears had significant and positive correlation with

weight of grain per ear at ≤0.005 level highlighting increasing any one of them would lead to improvement of other character. Significant negative correlation (at ≤ 0.005 level) observed for days to flowering with clum branching indicated increasing one character would lead to decrease in another character. Studied accessions grouped into four (04) main clusters exhibiting their genetic relatedness and which can be used in breeding and conservation programme of the crop.

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