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Morphological characterization of Foxtail millet germplasm

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ABSTRACT

Variability was studied among 246 foxtail millet [*Setaria italica* (L.) P.Beauv.] germplasm collected from different research stations and traditional farming villages of the Bangladesh. The collected genotypes were evaluated in the field at the Plant Genetic Resources Centre (PGRC), Bangladesh Agricultural Research Institute (BARI), Gazipur during winter 2013-2014 and 2014-2015. Qualitative variations were found in plant characters, leaf characters, inflorescence characters and seed characters. Erect and erect geniculate growth habit with non-pigmented, pigmented and deep purple plant was also exhibit among the accessions. Short Inflorescence lobes were present in maximum number of accessions followed by long lobes but no inflorescence lobes and large and thick inflorescence lobe was present in five and one accession respectively. Based on lobes compactness, medium was highest followed by loose, compact and spongy inflorescence lobe. Cylindrical, pyramidal, ovate to elliptic and ovate shaped with green and purple coloured inflorescence was present among the accessions. The majority of seed colour were white (58.1%), followed by black (13.4%) and others. Quantitative variations were observed among the accessions. Days to 50% flowering and days to mature seed harvest were 76 to 120 days and 116 to 157 days after sowing, respectively. Numbers of tiller per plant - ranged from 1 to 5.7. The highest coefficient of variation was found in number of tiller per plant (39.93 %) followed by peduncle length (35.59%). Days to seed harvest and days to 50% flowering showed the lowest (6.23% and 9.75%, respectively) coefficient of variation. The genotypes were grouped into ten clusters. The highest inter-cluster distance was observed between clusters VI and VII. Therefore, the genotypes belonging these clusters could be selected for future hybridization program.

Introduction

Small millets are the hardiest crops, belong to family Poaceae and include an estimated 8000 species belonging to some 600 genera. Among them, eight small seeded species are used as food crops in different countries globally. These include finger millet, foxtail millet, prosomillet,little millet, barnyard millet,kodo millet, teff and fonio millet. Foxtail millet is taken as a significant cereal since old times and has important role in development of human civilization in Asia and Europe (Li et al., 1996; Lu et al., 2009). Also, constituting the richness of different amino acids and nutritional minerals taken as food, it exhibits high photosynthetic efficiency and drought tolerance (Dai et al., 2008; Dai et al., 2011a).Their use as food, feed and fodder make them important for food security. Their grains are rich sources of calcium, iron, zinc, beta-carotene, and high quality proteins, contributing significantly in reducing malnutrition that affects nearly half of the world's population, particularly in developing countries of Africa and Asia (Gowda et al., 2002).

Foxtail millet has long been an important crop of the indigenous community. India ranks first in worldwide foxtail millet cultivation while Bangladesh ranks 46. Bangladesh has produced only 12 thousand tons foxtail millet from 38 thousand hectare of land in 2012 (FAOSTAT, 2013). In Bangladesh due to its greater drought tolerance and shorter growth period, only the indigenous people cultivated foxtail millet instead of rice. The ancient indigenous people cultivated it for various purposes, including for use in festivals and marriages. The stover serves as quality fodder for cattle. Foxtail millet has lost its importance as a food crop in competition with major cereals such as wheat, rice, maize and sorghum. Its short crop cycle and wide range of soil adaptability, it may remain a useful crop in Asia on poor agricultural land in regions with low rainfall or a short growing season. Plant genetic resources centre, BARI constitutes a rich and diverse collection of millet genotypes from different location of Bangladesh.

Assessment and utilization of genetic variability is very essential. The study of genetic diversity and genetic relatedness is necessary for crop improvement and in developing appropriate strategies for the conservation, exploitation and utilization of foxtail millet accession (Upadhya & Joshi, 2003). Understanding the pattern of diversity and the genetic structure of gene pools is critical for effective management and use of germplasm resources. Progress in plant breeding depends on identification of new sources of genetic variation for beneficial traits. Therefore, the experiment was conducted to characterize foxtail millet germplasm conserved at

Plant genetic resources center, BARI. Through identification of salient features, the promising accessions would be recommended to the growers.

Materials and Methods

Experimental site

The genotypes were evaluated in two consecutive years during winter, 2013-2014 and 2014-2015 at the PGRC, BARI Gazipur.

Plant materials

A number of foxtail millet germplasm were collected from government farms, markets and farm households from long period of time in PGRC, BARI. A total of 246 landraces were collected from different parts of Bangladesh. Special attention was given regarding variability among the accessions during collection so that we collect the different types of genotypes and covered total gene pool.

Experimental design

The field trials were in a randomized complete block design with two replications. Each accession was grown in four rows plot. Plot size was 3 m × 1.5 m. Seeds are sowing in a row maintaining distance 15 cm.

Cultural practices

BARI-recommended cultural practices were followed (Mondal et al., 2011). Irrigation was given at tillering, flowering and grain filling stage for ensuring proper growth and development of the crop. Weeding was also done as when required. Crop was reasonably free from any disease or insect damage and no chemical sprays were applied. Crop field was surrounded by repellents strip to protect the birds. Crops were harvested at their different maturity dates.

Data recording

Ten plants per replication were randomly chosen for biometric measurements. Plant data were measured by visual observation as a combination of active growth and plants appearing to be healthy and strong. All qualitative characters/traits were measured by a team following IBPGR (International Board for Plant Genetic Resources) descriptors for *Setaria italica* (IBPGR, 1985). Scoring of agro-morphological characters was done according to the procedures given in the descriptor. Quantitative data measurements were averaged from 10 randomly selected plants.

Statistical analysis

The data were analyzed using Genstat software (version 12.1.)

Descriptor list for foxtail millet

Growth habit: 1= Erect and 2= Erect geniculate, (Measured at vegetative stage)
Plant pigmentation: 0= Not pigmented (green), 3= Pigmented, and 7= Deep purple (Measured at vegetative stage)
Blade pubescence: 1= Essentially glabrous (Measured at vegetative stage)
Sheath pubescence: 1= Essentially glabrous (Measured at vegetative stage)
Ligule pubescence: 1= Essentially glabrous, 5 Medium pubescent and 9 Strongly pubescent (Measured at vegetative stage)
Degree of lodging at maturity: 1= Very slight, 2 = Medium (Ripening stage)
Senescence: 1= Actively growing (Ripening stage)
Inflorescence lobes: 0= Absent, 3= Short and 7= Long (At fully developed inflorescence)
Lobe compactness: 3= Loose, 5= Medium, 7= Compact, and 9= Spongy (At fully developed inflorescence)
Inflorescence bristles: 3= Short but obvious, 5= Medium, 7= Long and 9= Carrying a spikelet (At fully developed inflorescence)
Compactness of inflorescence: 3= Open 5= Medium, and 7= Compact (At fully developed inflorescence)
Spikelet arrangement: 3= Arranged around axis (At fully developed inflorescence)
Inflorescence colour: 1= Green, 2= Purple, and 3= Black (At fully developed inflorescence just before maturity)
Inflorescence shape: 1= Cylindrical, 3= Pyramidal, 5= Globose to elliptic, and 7= Obovate (At fully developed inflorescence)
Seed colour: 1= White, 3= Orange, 5= Black, and 7= Yellow (After threshing of seed)

Results and Discussions

Qualitative descriptor

a) Plant growth traits

Characterization of the qualitative traits revealed a wide variation among the accession (Table 1). The accessions varied considerably for growth habit and highest accessions showed erect type (229 accessions) but only seventeen accessions showed little different from erect. i.e. erect geniculate. Based on plant pigmentation, accessions were classified into three classes: Non pigmented or green, pigmented and deep purple. The majority were green (95.5%), followed by pigmented (4.1%), and deep purple (0.40%). About 93.9% accessions' leaf colour had green but the rest had pigmented among the accessions (Table 1). According to Reddy et al. (2006), among the 1535 foxtail millet germplasm of the ICRISAT petancheru genebank, plant and leaf colour in majority accession was green (74.6%) followed by pigmented and deep purple. Similarly plant growth habit was erect in maximum accession than decumbent, erect geniculate and prostate. No variation was observed in blade pubescence, sheath pubescence and senescence. Very slight (205 accessions) to medium (41 accession) lodging occurred among the accessions.

b) Inflorescence characters

Inflorescence characters showed great variation the collected foxtail millet accessions (Table 1). Based on Inflorescence lobes- short Inflorescence lobes were present in maximum number (160) of the accessions followed by long lobes (80 accessions) but no inflorescence lobes and large and thick inflorescence lobe was present in five and one accession respectively. Inflorescence bristle were categorized into four groups- medium s inflorescence bristles were the highest (97 accessions) followed by short but obvious (65 accessions) and long (59 accessions). However very short (24 accessions) and carrying spikelet (1 accession) inflorescence bristle also exists among the accessions. Based on lobes compactness, accessions were classified into four classes: loose, medium, compact and spongy. Medium compact lobe was highest (122 accessions) followed by loose inflorescence lobes (68 accessions) but compact (50 accessions) and spongy lobes (6 accessions) also appeared under the studied germplasm. Inflorescence compactness were medium in 126 accessions followed by open (76 accessions) while compact inflorescence also found among the accessions (44 accessions).

Spikelet arrangements were around the axis in all accessions. Maximum number of accessions (192 and 190) showed green coloured and cylindrical shape inflorescence followed by purple colour with pyramidal shape inflorescence (33 and 44 accessions, respectively), but globose to elliptic shape (6 accessions) and obovate shape (6 accessions) were also found among the foxtail millet germplasm. We observed wide variations of seed colors. Based on seed color, accessions were classified into five classes: white, orange, yellow, light yellow and black. The majority of seed colour were white (58.10%), followed by black (13.4%), yellow (12.6%), orange (11.8%) and light yellow (4.1%). Reddy et al., (2006) stated that, among the ICRISAT foxtail millet germplasm, majority of the accessions had long bristles, followed by medium bristles and short bristles. Of the three types of lobe compactness, compact lobes were predominant (64.4%), followed by medium (32.8%). Also observed a wide range of grain colors from black, combination of black and white, dark grey, light grey, red to yellow. Most of the studied traits both qualitative and quantitative were showed extensive variability among accessions is probably attributed to the genetic differences and also due to the environment from which they were collected (Krishnamurthy et al., 2014). Qualitative character's of individual accessions' has given in Table S1.

Quantitative descriptor

Range, mean, standard deviation and coefficient of variations were shown in Table 2. Days to 50 % flowering and days to mature seed harvest were 76 to 120 days and 116 to 157 days after sowing, respectively. Longer grain filing period is a desirable combination that the breeders are interested to find but the accessions that matured late might have experienced heat stress during grain filling and therefore suffered from yield loss. On an average, leaf blade length, leaf blade width and leaf sheath length were 38.9 cm, 2.4 cm and 14.7 cm, respectively. Plant height reached from 66.5 to 155.0 cm. Peduncle length was 4.3 to 34.8 cm and average inflorescence length was 19.2 cm among the accessions. Number of tiller per plant ranged from 1 to 5.7. The highest coefficient of variation was found in number of tiller/ plant (39.93%) followed by peduncle length (35.59%) and coefficient of variation in inflorescence length were also high (23.64%). Days to seed harvest and days to 50% flowering showed the lowest (6.23% and 9.75%) coefficient of variation. Considering the above characteristics some of accessions (BD-862, BD-867, BD-901, BD-940, BD-950, BD-951, BD-1067 and BD-1094) has been shown batter performance.

Principal component analysis (PCA)

Multivariate analysis of the accessions revealed that the first three Principal Components (PC1 to PC 3) gave eigen- values > 1 and cumulatively accounted for 70.3% of the total variation (Table 3). The first PC axes accounted for 32.9% of the total multivariate variation, while the second accounted for 23.0% and the third for 14.4%. The cumulative proportion of the variation reached 70.3% in the first three PC axes and 81.6% in the first four axes. The high degree of variation in first three PC axes indicates a high degree of variation of these characters. Though there are no guidelines to determine the significance or importance of a coefficient, which is eigen– vactor (Duzyaman, 2005). However higher coefficients for a certain trait indicate the relatedness of the trait to respective PC axes (Sneath & Sokal, 1973). Characters with high coefficients in the PC1 to PC3 should be considered as more important since these axes explain more than half of the total variation. The variation in PC1 was mainly associated with plant height and numbers of tiller per plant, in PC2 with leaf blade length, leaf sheath length, peduncle length and in PC3 inflorescence length.

Table 1. Qualitative variation of different descriptors in foxtail millet accessions

Name of descriptor	Descriptor state	No. of accession (s)	Frequency (%)
Growth habit	Erect	229	93.1
	Erect geneculate	17	6.9
Plant pigmentation	Not pigmented	235	95.5
	Deep purple	1	0.4
	Pigmented	10	4.1
Leaf colour	Green	231	93.9
	Pigmented	15	6.1
Blade pubescence	Essentially glabrous	246	100
Sheath pubescence	Essentially glabrous	246	100
Degree of lodging at maturity	Very slight	205	83.3
	Medium	41	16.7
Senescence	Actively growing	246	100
Inflorescence lobes	Absent	5	2.0
	Short	160	65.0
	Long	80	32.6
	Large and thick	1	0.4
Inflorescence bristles	Very short	24	9.8
	Short but obvious	65	26.4
	Medium	97	39.4
	Long	59	24.0
	Carrying spikelet	1	0.4
Lobes compactness	Loose	68	27.7
	Medium	122	49.6
	Compact	50	20.3
	Spongy	6	2.4
Inflorescence compactness	Open	76	30.9
	Medium	126	51.2
	Compact	44	17.9
Inflorescences colour	Green	192	78.0
	Purple	33	13.4
	Black	21	8.6
Inflorescences shape	Cylindrical	190	77.2
	Pyramidal	44	18.0
	Globose to Elliptic	6	2.4
	Obovate	6	2.4
Seed colour	White	143	58.1
	Orange	29	11.8
	Yellow	31	12.6
	Light yellow	10	4.1
	Black	33	13.4

Table 2. Quantitative variation of different descriptors in foxtail millet accessions

Descriptors	Range	Mean	Sd	CV (%)
Plant height (cm)	66.5 -155.0	115.6	19.99	17.30
Leaf blade length (cm)	24.0 - 51.0	38.9	5.44	13.97
Leaf blade width (cm)	1.80 - 4.0	2.4	0.37	15.45
Leaf sheath length (cm)	11.0 - 20.	14.7	1.82	12.40
Inflorescence length (cm)	6.0 - 30.4	19.2	4.55	23.64
Peduncle length(cm)	4.3 - 34.8	17.7	6.29	35.59
Number of tiller per plant	1.0 - 5.7	3.0	1.19	39.93
Day to 50% flowering	76 - 120	98.0	9.56	9.75
Days to seed harvest	116 - 157	135.0	8.41	6.23

Table 3. Extraction of eigen vectors, eigen values, percent of variation and cumulative variations for principal components of foxtail millet accessions

	PC 1	PC 2	PC 3	PC 4
Eigen value	2.302	1.607	1.005	0.793
Explained proportion of variation (%)	32.9	23.0	14.4	11.3
Cumulative proportion of variation (%)	32.9	55.9	70.3	81.6
Variables	Eigen- vectors			
Plant height (cm)	0.97976	-0.10191	-0.15918	0.06581
Leaf blade length (cm)	0.01722	-0.69662	0.65449	0.23846
Leaf blade width(cm)	0.0127	-0.01071	0.01836	0.0027
Leaf sheath length (cm)	0.10854	-0.14232	0.09907	0.01062
Numbers of tiller per plant (no.)	0.98792	-0.06833	-0.0814	-0.00411
Peduncle length (cm)	0.15977	-0.69229	-0.64536	-0.25829
Inflorescence length (cm)	0.11778	0.00495	0.3362	-0.9337

PCA analysis indicate that plant height, number of tiller plant, leaf blade length, leaf sheath length, peduncle length and inflorescence length were among the most important descriptors which accounted for more than 70% of the phenotypic variation expressed in this germplasm collection. These descriptors were therefore found to be most useful for studying the variability of the population. It is suggested that the use of these characters will save considerable amount of time for identification of foxtail millet germplasm. Successful breeding of high yielding varieties depends on the yield contributing morphological traits and choosing small number of important traits having positive correlation. Flag leaf area, plant height, peduncle length and number of tillers per plant are an important morphological yield contributing traits that are positively correlated with yield per plant (Khaliq et al., 2008). The present study also suggested that high yielding accessions of foxtail millet may be selected by indirect selection of plant height, leaf blade length, leaf sheath length, peduncle length, inflorescence length, and number of tiller plant character containing accessions.

Cluster analysis

Hierarchical clustering of the 246 foxtail millet germplasm based on quantitative characters was carried out. A dendrogram using all clusters in vertical orientation was determined using between groups linkage measured in squared Euclidean distance (Fig. 1a and 1b). The population was grouped in ten major clusters (Table 4). Cluster V was the largest with sixty seven accessions followed by sixty two accessions in cluster IV and fifty two accessions in cluster I. cluster II, cluster III, cluster VII and cluster VIII consisted of seven, twenty seven, seventeen and three accessions, respectively. Remaining clusters (cluster VI, cluster IX and cluster X) was found with only two accessions each.

Canonical variate analysis (CVA**)** was done to compute the inter-cluster distances (Table 6). The highest inter-cluster distance was observed between clusters VI and VII (17.44), followed by the distance between clusters II and VII (15.63), V and VI (13.91). In contrast, the lowest inter-cluster distance was observed between cluster IV and IX (1.34), followed by cluster I and IX (1.86). However, the maximum inter-cluster distance was observed between the clusters clusters VI and VII (17.44) indicating that genotypes from these two clusters, if involved in hybridization, may produce a wide spectrum of segregating population (Dhillon et al., 1999). It is assumed that maximum amount of heterosis will be manifested in cross combination involving the genotypes belonging to most divergent clusters. According to the cluster means, Cluster VII showed better performance in case of number of tiller plant, peduncle length and inflorescence length (Table 5). Thus indicates that genotype of this cluster could be used for parent in future hybridization program for higher seed yield.

Table 4. Number of cluster and genotypes under each cluster of foxtail millet accessions

Cluster No.	**Cluster member**								
Cluster I	BD-860	BD-871	BD-875	BD-876	BD-878	BD-886	BD-888	BD-892	BD-904
	BD-908	BD-916	BD-924	BD-926	BD-928	BD-931	BD-940	BD-942	BD-945
	BD-947	BD-950	BD-952	BD-953	BD-955	BD-956	BD-961	BD-967	BD-984
	BD-1000	BD-1004	BD-1005	BD-1008	BD-1020	BD-1021	BD-1022	BD-1035	BD-1044
	BD-1053	BD-1059	BD-1065	BD-1067	BD-1074	BD-1088	BD-1095	BD-1098	BD-1099
	BD-1100	BD-1191	BD-1208	BD-1221	BD-1225	BD-1262	IA-48		
Cluster II	BD-862	BD-897	BD-898	BD-929	BD-934	BD-1045	BD-1101		
Cluster III	BD-867	BD-881	BD-890	BD-891	BD-893	BD-900	BD-901	BD-903	BD-905
	BD-927	BD-930	BD-932	BD-935	BD-936	BD-943	BD-944	BD-948	BD-949
	BD-951	BD-959	BD-1019	BD-1028	BD-1033	BD-1049	BD-1057	BD-1077	BD-1094
	BD-1230								
Cluster IV	BD-877	BD-879	BD-885	BD-887	BD-910	BD-911	BD-913	BD-921	BD-938
	BD-941	BD-1016	BD-1063	BD-1066	BD-1068	BD-1093	BD-1150	BD-1154	BD-1158
	BD-1161	BD-1168	BD-1176	BD-1188	BD-1189	BD-1196	BD-1198	BD-1207	BD-1209
	BD-1217	BD-1234	BD-1238	BD-1239	BD-1240	BD-1242	BD-1243	BD-1244	BD-1245
	BD-1246	BD-1248	BD-1257	BD-1259	BD-1263	BD-1267	BD-1268	BD-1269	BD-1276
	BD-1283	BD-1287	BD-1290	BD-1293	BD-1310	BD-1313	BD-1314	BD-1317	BD-1325
	BD-1327	BD-1330	AM-21	NIR-89	AIR-04	IA-27	IA-30	RAI-59	
Cluster V	BD-880	BD-1007	BD-1012	BD-1114	BD-1115	BD-1116	BD-1127	BD-1134	BD-1142
	BD-1143	BD-1144	BD-1157	BD-1165	BD-1167	BD-1169	BD-1171	BD-1179	BD-1194
	BD-1200	BD-1202	BD-1206	BD-1214	BD-1220	BD-1222	BD-1227	BD-1235	BD-1237
	BD-1241	BD-1251	BD-1252	BD-1254	BD-1255	BD-1258	BD-1264	BD-1270	BD-1273
	BD-1277	BD-1278	BD-1279	BD-1280	BD-1281	BD-1284	BD-1285	BD-1286	BD-1289
	BD-1294	BD-1295	BD-1301	BD-1305	BD-1306	BD-1309	BD-1311	BD-1312	BD-1321
	BD-1324	BD-1328	RISA-36	RISA-42	RISA-77	RISA-87	AI-52	AI-53	AI-54
	AM-02	AM-23	AM-54	IA-57					
Cluster VI	BD-899	BD-1032							
Cluster VII	BD-1105	BD-1129	BD-1130	BD-1213	BD-1215	BD-1216	BD-1249	BD-1271	BD-1272
	BD-1275	BD-1288	BD-1291	BD-1296	RISA-92	RISA-123	AI-10	KASI-61	
Cluster VIII	BD-1219	BD-1292	AR-33						
Cluster IX	BD-1229	BD-1307							
Cluster X	BD-1231	BD-1261							

Table 5. Cluster means for 7 quantitative characters in 246 foxtail millet accessions

Parameters	Cl-I	Cl-II	Cl-III	Cl-IV	Cl-V	Cl-VI	Cl-VII	Cl-VIII	Cl-IX	Cl-X
Plant height (cm)	160.93	145.54	132.3	126.3	113.47	116.37	103.38	115.82	80.83	93.85
Leaflet length (cm)	36.22	40.84	41.42	36.54	42.93	30.74	40.41	40.83	39.14	36.22
Leaflet width (cm)	2.38	2.44	2.38	2.36	2.63	2.23	2.42	2.49	2.44	2.35
Leaf sheath length	14.67	14.78	15.43	14.58	14.98	13.17	15.01	14.83	14.42	14.29
Numbers of tiller/ plant	2.44	2.31	2.02	2.39	2.52	3.02	4.02	3.32	3.15	3.41
Peduncle length (cm)	14.37	12.6	11.93	12.11	12.57	16.3	24	21.49	17.74	22.91
Inflorescence length (cm)	22.03	23.78	17.48	18.78	20.91	19.23	24.36	16.85	16.31	16.85

Table 6. Inter cluster distances of ten clusters from 246 foxtail millet accessions

Cluster number	I	II	III	IV	V	VI	VII	VIII	IX	X
I	0									
II	6.32	0								
III	3.45	2.86	0							
IV	3.17	9.19	6.41	0						
V	6.10	12.18	9.39	2.98	0					
VI	8.21	2.00	4.79	10.95	13.91	0				
VII	9.36	15.63	12.79	6.50	3.69	17.44	0			
VIII	6.01	11.22	8.70	3.16	2.78	12.70	6.10	0		
IX	1.86	7.88	5.07	1.34	4.32	9.67	7.77	4.2	0	
X	4.23	8.36	6.06	3.07	5.01	9.74	8.68	3.01	3.03	0

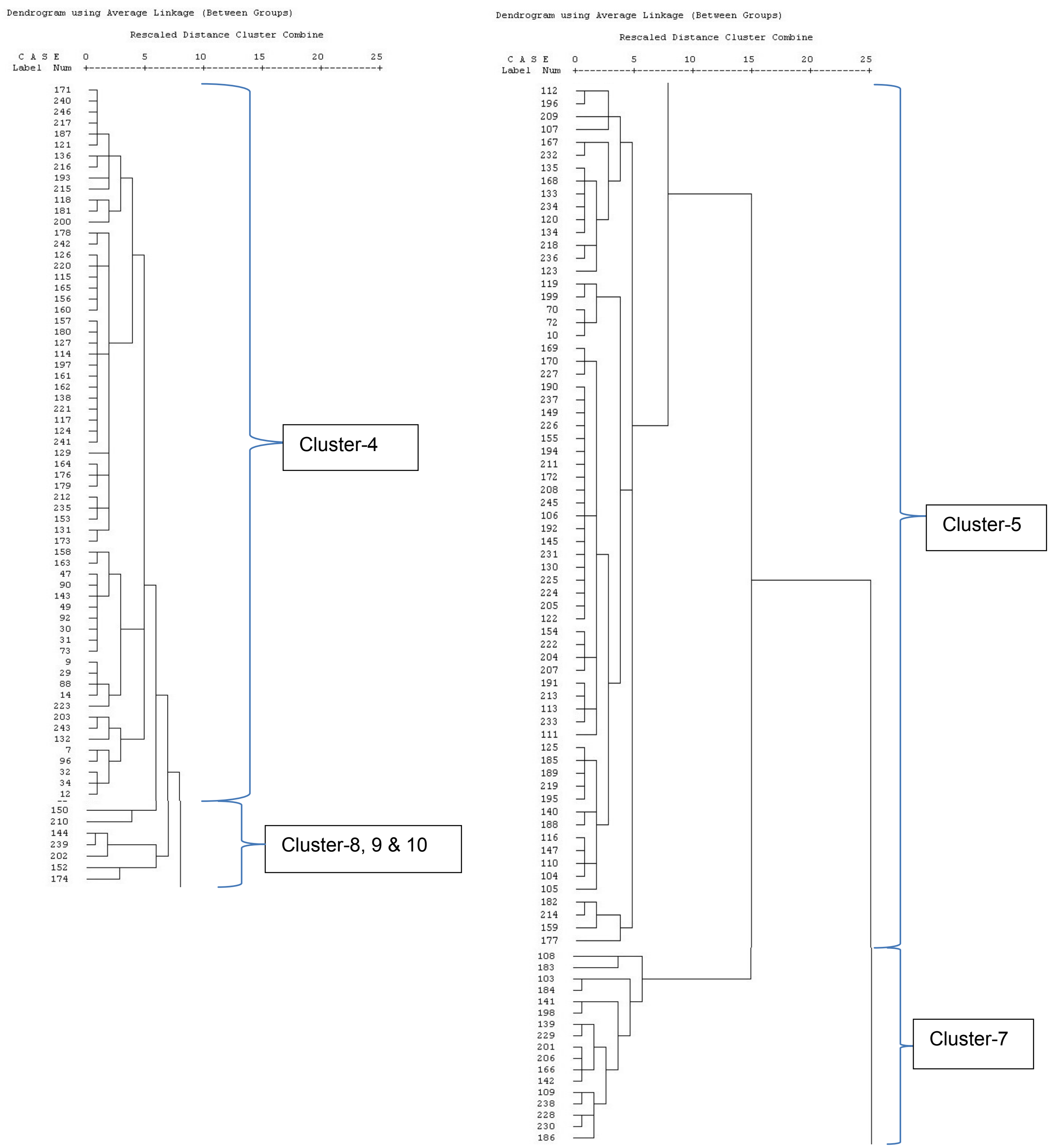

Figure 1a. Dendogram showing position of cluster of the 246 foxtail millet accessions

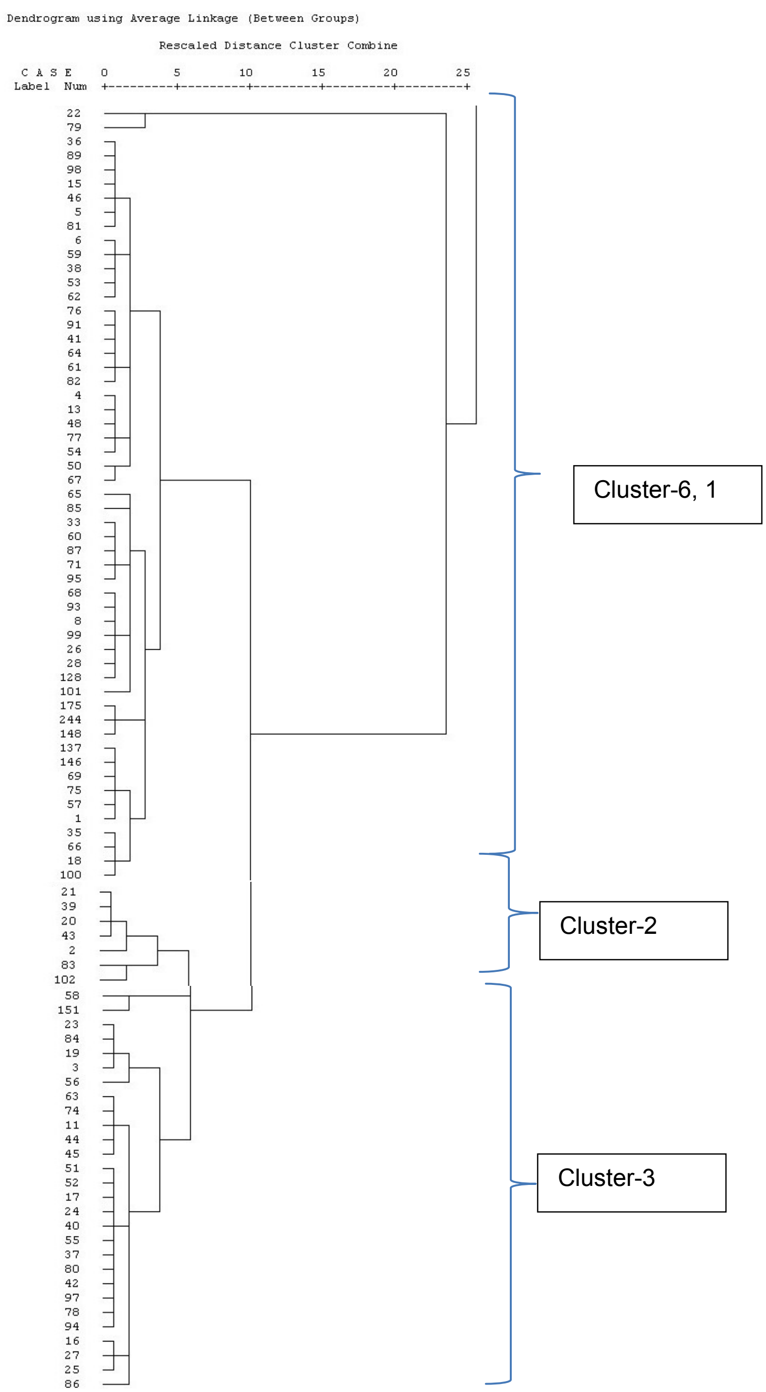

Figure 1b. Dendogram showing position of cluster of the 246 foxtail millet accessions

Conclusion

Study on 246 foxtail millet accessions exhibit variations considering both the qualitative and quantitative characters. Qualitative characters like growth habit, plant pigmentation, inflorescence colour, inflorescence shape and compactness and seed colour were differ from each other among the accessions. Plant height, number of tiller per plant, inflorescence length, peduncle length, leaf blade length and leaf blade width also express variation among the accessions. Days to 50% flowering and days to harvest also different from one to another. This variability can be used in varietal improvement of foxtail millet. Phenotypic variation of the foxtail millet accessions was found to be related to the diverse geographic origins.

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