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Research Article

Characterization of Barnyard millet *(Echinocloa frumentaceae* (Roxb.) Link) germplasm for quantitative traits to enhance its utilization

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Abstract
Barnyard millet, *Echinochloa* sp. is one of the important small millet crops used as food and fodder crop. Utilization of diverse germplasm is important in breeding programme to improve yield of the crop. In the present study, 494 barnyard millet germplasm were characterized using multivariate traits. Wide variation was observed for most of the traits indicated the scope of importance of these characters by direct selection. Phenotypic correlation between grain yield per plant was in significantly positive association with days to 50% flowering, plant height, the number of productive tillers, days to maturity and straw weight whereas, it was negatively associated with length of peduncle. Principle component analysis showed that the first two components contributed about 64% of the total variability. The proportions of the total variance attributed to the first two principle components were 43% and 20% respectively. The characters days to 50% flowering, plant height, days to maturity and straw weight were the most important traits contributing to the total variance in the first two principle components, indicating their importance for the characterization of barnyard millet germplasm accessions and these traits could be effectively used for future breeding programmes to create more variability. K means clustering method based on 7 quantitative traits among 494 barnyard millet germplasm was able to separate and grouped them into 23 clusters. Crossing accessions belonging to different clusters would maximize opportunities for transgressive segregation because of the higher probability that unrelated genotypes will contribute unique desirable alleles at multiple loci. Variability present in barnyard millet germplasm can be effectively used for the selection of specific donor lines for improvement of barnyard millet.

Key words
Barnyard millet, Phenotypic correlation, Principle component analysis, quantitative traits

INTRODUCTION

Barnyard millet, *Echinochloa* sp. (2n=36, 54) is a self-pollinating crop which belongs to the family Poaceae and is grown as cereal or fodder crop. Barnyard millet is the fastest growing crop among all millets and can be harvested in a short period of nine weeks. Barnyard millet is mostly grown in India, China, Japan, and Korea for human being consumption and fodder (Upadhyaya *et al*., 2014). The crop is valued for its drought tolerance (Dwivedi *et al*., 2012), short growth period and superior nutrition value (Saleh *et al*., 2013). In India, the crop is grown in Madhya Pradesh, Uttarakhand, Tamil Nadu, Andhra Pradesh, Karnataka, Maharashtra and Bihar. Barnyard millet has emerged as very imperative dual purpose nourishes and feed crop. Its grains contains of carbohydrate (65%), protein (11%), crude fiber (13.6%) and fat (3.9%) (Hadimani *et al.,* 1993).It is also an excellent source of minerals such as Iron (Fe), and Zinc (Zn) and antioxidant compounds (Watanabe, 1999).

Information about germplasm diversity and genetic relationships among accessions or cultivars is of fundamental importance for breeding and the management of germplasm. It can support breeding activities by both farmers and plant breeders (Govindaraj *et, al*., 2015).

Principal Component Approach (PCA) and cluster analysis serve as potential tools in evaluating the phenotypic diversity. It is very helpful in deciding which traits of crop contributing most to yield. Subsequently these agronomic traits should be emphasized in the breeding programme. Cluster analysis identifies and classifies objects individuals on the basis of similarity of characteristics they possess. It seeks to minimize within group variance and maximize between group variance. It is helpful for parental selection in breeding programme and crop modeling.

The National Gene-bank at ICAR-NBPGR, New Delhi holds the large number of barnyard millet germplasm accessions. A total of 494 barnyard millet accessions were used for evaluation and characterization in our present study, with an objective to characterize 494 accession of barnyard millet germplasm by means of quantitative traits to understand the association of various traits. PCA and clustering analysis which enable to classify them into distinct phenotypic groups based on their genetic variability.

MATERIALS AND METHODS

The experimental material for the present study comprised of 494 germplasm accessions of barnyard millet. These 494 germplasm accessions were collected from NBPGR, New Delhi.

Evaluation and characterization of these 494 accessions of barnyard millet germplasm and 3 check varieties were grown in augmented block design during *Kharif* 2016. The characterization site, Bengaluru located at 13^0 05” N latitude and 77^0 34” E longitudes. The center is at an altitude of 924 meters above the mean sea level. The annual rainfall ranges from 528 mm to 1374.4 mm with the mean of 915.8 mm. The germplasm accession were divided into 13 blocks, each consisted of 38 accessions and 3 check varieties namely VL 29, VL 172 and VL 207. Each accession was grown in a single row of 3 meter length, spaced 30cm apart, plant to plant spacing within the row was 10 cm. Crop was supplied with recommended dose of fertilizer of 10 kg N ha^{-1} and 20 kg P_2O_5 ha^{-1} as a basal dose and 10 kg N ha^{-1} at the time of earthing up. The experiment was provided with life saving irrigation and protected from weeds, pests and diseases.

Observations were recorded from 5 randomly selected plants in each accession for 7 quantitative characters *viz*., Days to 50% flowering, Plant height (cm), The number of basal tillers, Peduncle length (cm), Days to maturity, Straw weight (g) and Grain yield per plant (g).

Mean, Range, variance and standard deviation were worked out by adapting standard methods (Panse and Sukathme, 1964) using SPSS statistical package. Phenotypic correlation coefficients were calculated using the formula as suggested by Johnson *et al.* (1955). Phenotypic correlations were estimated among all traits in the entire germplasm and identified the number of significant and meaningful correlation. The data collected for all quantitative characters were subjected to analysis of variance for augmented block design according to the method suggested by Federer and Raghava Rao (1975).

Principal Component Analysis (PCA) was computed using WINDOWS STAT statistical package as suggested by Johnson and Wichern (1988). PCA was computed for seven quantitative traits to find out the relative importance of different traits in capturing the variation in entire germplasm. Seven quantitative traits were subjected to multivariate K means cluster (Lloyd, 1982) analysis using the software WINDOWS STAT.

RESULT AND DISCUSSION

Analysis of variance showed significant differences for all quantitative characters suggesting significant variability for all the traits. The mean, range and variance for all the seven quantitative traits are given in **table 1.** Days to 50 per cent of flowering was ranged from 32 (IC 404528) to 71(IC404450) days with mean value of 41.79. Accessions IC 404528, IC 404293 and IC 473046 were early to flower; hence these accessions could be utilized for developing an early duration varieties in barnyard millet.

Table 1. Pattern of genetic variability for 7 quantitative traits in 494 Barnyard millet germplasm

Characters	Mean	Range	Minimum	Maximum	Variance	Std. Deviation	Sem
Days to 50% flowering	41.79	39.00	32.00	71.00	39.49	6.28	0.28
Plant height (cm)	110.82	95.80	62.00	157.80	322.42	17.96	0.81
Number of productive tillers	4.18	10.40	2.00	12.40	2.97	1.72	0.08
Length of peduncle (cm)	5.77	10.60	2.20	12.80	4.24	2.06	0.09
Days to Maturity (in Days)	84.79	39.00	75.00	114.00	39.49	6.28	0.28
Straw weight(g)	39.88	79.80	16.20	96.00	208.91	14.45	0.65
Grain yield (g/plant)	10.42	33.80	4.20	38.00	23.85	4.88	0.22

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In the entire germplasm IC 472843 showed the lowest plant height of 62cm and IC404413 showed the highest plant height of 157 cm. Accessions IC472993, IC404288 and IC404551 recorded more number of basal tillers of 12.40, 11.60 and11.60 respectively in the entire set of germplasm. Similarly, the character grain yield per plant ranged from 4.20g (IC404550) to 38g (IC 52699) with a mean yield of 10.42g. Accessions IC 52699(38gm), IC52701 (35g) and IC 601276 (33g) showed the maximum grain yield. Straw weight ranged from 16.20g (IC473093) to 96g (IC404342 and IC404382) with a mean of 39.88g. This variation in majority of the characters can be exploited in future for the selection of germplasm with dwarf types, early flowering, more number of tillers, high yielding types. Similarly this germplasm can be used as parent for hybridization to improve some specific characters in released varieties. (Sood *et al*, 2015 and Manimekalai *et al*., 2018). Top 20 accessions of barnyard millet germplasm identified for their superior agronomic traits are presented in **table 2.**

Table 2. Top 20 accessions of Barnyard millet identified for their superior agronomic traits

Sl No.	High yielding accessions		Early flowering accessions		Dwarf accessions		Accessions with more number of tillers		Accessions with long peduncle length		Straw weight	
	National ID	Grain yield (g/ plant)	National ID	Days to 50% flowering	National ID	Plant height (cm)	National ID	Number of productive tillers	National ID	Length of peduncle (cm)	National ID	Straw weight(g)
1	IC52699	38.00	IC404528	32.00	IC472843	62.00	IC472993	12.40	IC472678	12.80	IC404342	96.00
2	IC52701	35.00	IC404293	32.00	IC472638	64.40	IC404288	11.60	IC404301	12.60	IC404382	96.00
3	IC601276	33.00	IC473046	32.00	IC404388	64.80	IC404551	11.60	IC473017	12.00	IC404323	95.40
4	IC472608	33.00	IC404529	33.00	IC472993	70.80	IC404313	11.00	IC472881	11.80	IC472920	90.00
5	IC404396	31.00	IC404292	33.00	IC473120	72.20	IC473102	11.00	IC473073	11.40	IC404446	84.00
6	IC601274	31.00	IC404302	33.00	IC472663	72.20	IC404528	10.80	IC472692	11.40	IC473129	84.00
7	IC404382	27.20	IC404303	33.00	IC472585	72.80	IC404384	10.20	IC404532	11.08	IC404380	84.00
8	IC404406	26.80	IC404313	33.00	IC473105	74.40	IC404317	9.40	IC404295	11.00	IC404413	84.00
9	IC472934	25.80	IC404524	34.00	IC472698	75.00	IC404550	9.40	IC472724	11.00	IC472732	84.00
10	IC601275	24.60	IC404531	34.00	IC601276	75.20	IC473140	9.20	IC472876	11.00	IC404385	78.00
11	IC472715	23.25	IC404532	34.00	IC472492	75.60	IC404539	9.00	IC404303	10.80	IC472958	78.00
12	IC473003	23.20	IC404536	34.00	IC472523	77.00	IC404305	8.60	IC473029	10.80	IC472901	75.60
13	IC472527	22.80	IC404290	34.00	IC472934	78.00	IC404545	8.60	IC404317	10.70	IC404378	72.00
14	IC472881	22.80	IC404294	34.00	IC473125	78.00	IC472959	8.60	IC404531	10.40	IC404384	72.00
15	IC404381	22.40	IC404297	34.00	IC472778	78.20	IC473031	8.60	IC473070	10.40	IC404412	72.00
16	IC472778	22.40	IC404307	34.00	IC472826	78.80	IC404474	8.40	IC472975	10.40	NC59226	72.00
17	IC52691	22.00	IC404317	34.00	IC472580	80.40	IC404548	8.40	IC404296	10.00	IC404449	69.60
18	IC472989	21.40	IC404550	34.00	IC52701	80.80	IC472955	8.40	IC473040	10.00	IC404314	69.60
19	IC52695	21.00	IC472886	34.00	IC473070	82.20	IC404541	8.20	IC472920	10.00	IC473100	69.60
20	IC472876	21.00	IC473006	34.00	IC472739	83.00	IC404553	8.20	IC404550	9.80	IC404310	69.00

Grain yield is a complex character influenced by large number of other component characters. Character association may be used to identify a few traits that are less relevant and could be of low priority in germplasm evaluation. This helps in improving the efficiency of selection. The correlation between the characters may exist due to various reasons such as pleiotropy, genetic linkage or association of loci or blocks of loci governing variability for different characters located on same chromosome. Correlation coefficient between traits is presented in **table 3**.

Among the 7 morphological traits studied, all the traits *viz*., days to 50% flowering (0.18), plant height (0.11), the number of productive tillers (0.18), days to maturity (0.16) and straw weight (0.30) showed a significant positive association with grain yield per plant except length of peduncle, which showed a negative association (-0.13) with grain yield at P=0.01 level. Earlier studies have also reported significant positive association of grain yield per plant with component characters (Mehta *et al*, 2005; Joshi V, 2013 and Sood *et al*, 2015). This positive correlation for yield with other characters indicated that all these characters could be simultaneously improved and it is also suggested that increase in any one of them would lead to improvement of other character. Hence, the selection criteria should consider all these characters for the improvement of grain yield in barnyard millet.

Significant negative correlation observed for length of peduncle (-0.13) with grain yield per plant (P=0.01) indicated that increase in one character would lead to decrease in another character.

PCA was applied as a reductionist approach of the multivariate data to measure the importance and contribution of each component to the total variance. PCA provide 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 the importance of the first two principal components in discriminating the entire set of barnyard millet germplasm. The percentage of variation explained by the first two principal components and the vector loadings for each agronomic character and principal components are shown in **table 4**.

The percentage of total variation explained by two principal components was 64%. The PC1, the most important coordinate accounted for 43% in the entire germplasm. PC1 separates accessions mainly on five traits (Days to 50% flowering, plant height, days to maturity, straw weight and grain yield per plant). Days to 50% flowering (0.94) and days to maturity (0.94) contributes more to the variation followed by plant height (0.69), straw weight (0.68) and grain yield per plant (0.31) had the highest loadings in PC1 indicating their significant importance for these components. These traits had the largest participation in the divergence and carried the largest portion of its variability. All other characters contributed negative to the first component.

Table 3. Phenotypic correlation co-efficient between 7 quantitative traits in 494 Barnyard millet germplasm

Traits	Days to 50% flowering	Plant height (cm)	Number of productive tillers	Length of peduncle (cm)	Days to Maturity (in Days)	Straw weight(g)	Grain yield (g/plant)
Days to 50% flowering	1.00	0.61**	-0.22*	-0.30*	1.00	0.49**	0.18**
Plant height (cm)		1.00	-0.03	0.14*	0.61**	0.47**	0.11**
Number of productive tillers			1.00	0.41**	-0.22**	-0.01	-0.18**
Length of peduncle (cm)				1.00	-0.30**	-0.14*	-0.13*
Days to Maturity (in Days)					1.00	0.49**	0.16*
Straw weight(g)						1.00	0.30**
Grain yield (g/plant)							1.00

Table 4. Vector loadings and percentage variation explained by first two principal components in 494 Barnyard millet germplasm

	PC1	PC2
Eigen value	3.05	1.45
Variance explained (%)	0.43	0.20
Cumulative variance explained (%)	0.43	0.64
Eigen vectors		
Days to 50% flowering	0.94	0.05
Plant height (cm)	0.69	0.51
Number of productive tillers	-0.31	0.70
Length of peduncle (cm)	-0.36	0.74
Days to maturity	0.94	0.55
Straw weight per plant (g)	0.68	0.19
Grain yield per plant (g)	0.31	-0.32

The second principal component (PC2) contributed 20 percent to the total variation. length of peduncle (0.74) contributed more to the total variation followed by the number of productive tillers (0.70), days to maturity (0.55), plant height (0.51) and straw weight (0.19) while, grain yield per plant contributed negatively.

Considering the principal component in entire germplasm, four traits (days to 50% flowering, plant height, days to maturity and straw weight) explained more variance in the first two principal components, indicating their importance for the characterization of barnyard millet germplasm accessions and these traits could be effectively used for future breeding programmes to create more variability. Characters with high variability are expected to provide high level of transgressive segregation in breeding populations. This is important for breeder to investigate high yielding, early maturing and dwarf varieties through conventional breeding. Similar findings with regard to contribution of characters to total variance, Salini *et al.* (2010) in proso millet observed grain yield per plant, plant height, days to flowering and productive tillers per plant were contributed more for variance. Layton and Kellogg (2014) reported a first principal component and explained 17% of the variation in *Setaria viridis*. Azad *et al*. (2012) reported traits such as grain yield per plant and plant height contributed more for total variance.

K means cluster analysis based on quantitative traits divided 494 barnyard millet germplasm into 23 clusters **(table 5**). Clustering pattern could be utilized in choosing the diverse genotype which is likely to generate the highest possible variability for various economic characters. Cluster 17 comprising of 34 accessions which are mostly characterized by dwarf plants and early maturing types. Cluster 19 was the largest cluster comprised of 35 accessions which were characterized by dwarf plants, early

Table 5. Number of accessions constituted in 23 clusters of494Barnyard millet germplasm

Cluster number	No. of Accessions in the cluster	Cluster number	No. of Accessions in the cluster
1	27	13	2
2	27	14	28
3	30	15	27
4	12	16	31
5	17	17	34
6	30	18	21
7	18	19	35
8	25	20	15
9	11	21	18
10	16	22	18
11	6	23	31
12	16		

Table 6. The average of 7 quantitative traits for each cluster in 494 Barnyard millet germplasm accessions

Cluster number	Days to 50% flowering	Plant height (cm)	No. of basal Tillers	Length of peduncle	Days to maturity	Straw weight per plant (g)	Grain yield per plant (g)
1	34.37	102.23	6.26	7.40	77.37	31.58	6.65
2	39.07	112.53	3.80	6.40	82.07	37.86	10.51
3	36.53	113.91	5.17	7.92	79.53	32.96	7.40
4	44.33	144.78	4.72	7.45	87.33	53.45	8.35
5	54.59	148.00	4.46	5.28	97.59	57.53	11.04
6	55.73	133.40	3.57	4.54	98.73	55.73	13.13
7	49.00	121.57	3.41	4.51	92.00	52.61	11.33
8	40.64	120.42	4.31	7.00	83.64	38.52	10.26
9	37.36	130.58	4.69	7.87	80.36	42.71	7.49
10	39.44	74.06	3.80	4.41	82.44	35.00	12.85
11	50.17	101.47	3.73	3.57	93.17	57.60	15.34
12	35.13	87.43	5.41	6.34	78.13	32.37	7.22
13	50.00	72.80	4.50	3.70	93.00	53.10	31.00
14	42.29	104.06	3.42	4.77	85.29	35.99	12.33
15	38.19	91.58	4.03	5.03	81.19	31.22	9.62
16	38.45	100.39	3.90	4.81	81.45	34.08	10.76
17	38.59	105.27	3.82	5.64	81.59	34.70	9.01
18	41.62	98.03	3.44	4.71	84.62	34.83	11.45
19	36.57	96.98	4.85	6.13	79.57	31.31	7.73
20	42.00	89.25	3.64	4.55	85.00	39.84	21.62
21	43.67	127.80	3.58	6.98	86.67	45.44	10.52
22	44.72	113.29	2.98	5.22	87.72	45.87	14.50
23	47.87	135.84	4.23	5.69	90.87	48.99	9.98

duration and low yielding accessions. Apart from cluster 19 and 17 large numbers of accessions were grouped in clusters 16, 23, 3, and 6 with 31, 31, 30 and 30 accessions respectively. Cluster 13, 11 and 9 had less number of accessions of 2, 6, and 11 respectively. This indicated that accessions in these clusters have wide diversity for various characters. Hybridization using genotypes belong to clusters 2, 6, and 11 might be used for exploitation of hybrid vigor. Similar results have been reported by Salini *et al*. (2010), in which the proso millet genotypes used were grouped into 17 clusters. Manimekalai *et al*., 2018, grouped 61 genotypes of barnyard millet germplasm in 13 clusters based on the Bouches clustering technique. Sood *et al*., (2015) grouped 95 germplasm accessions of barnyard millet into two groups *viz*., Group A comprised of 43 accessions and Group B comprised of 51 accessions using two way cluster analysis.

Cluster means for all seven quantitative traits are shown in **table 6**. The accessions in the cluster 14 showed more mean grain yield followed by cluster 20, 11 and 22 whereas genotypes in cluster 3, 9, and 19 were having less mean grain yield.

Accessions which are present in these diverse clusters can be utilized for hybridization to get transgressive segregants for that trait, which could be used for developing superior high yielding varieties. Accessions which are present in the clusters 10, 12 and 20 comprised of dwarf types. These accessions could be utilized in developing dwarf varieties in barnyard millet. Cluster 1 and cluster 12 comprised accessions with more number of basal tillers. These accessions could be utilized in developing high yielding types as number of productive tillers is positively correlated with grain yield. Likewise, genotypes in diverse clusters could be utilized in hybridization programme to develop high yielding, dwarf and early duration types in barnyard millet.

Assessment of genetic diversity and relationship among various accessions is fundamental importance for efficient preservation and to exploit the barnyard millet genetic resources. Multivariate statistical analysis provides a means for estimating morphological diversity between germplasm accessions. These tools are useful for the evaluation of potential breeding value of germplasm. The PCA and cluster analysis provided a simplified classification of barnyard millet germplasm accessions for use in breeding. In PCA traits days to 50% flowering, plant height, days to maturity and straw weight were found as more important for characterizing barnyard millet germplasm, as these traits explained more variation. Days to 50% flowering and the number of productive tillers could be indirectly used for selection as these traits are positively correlated. Categorizing germplasm accessions into morphologically similar and presumably genetically similar groups is useful for selecting parents for crossing (Souza *et al*., 1991). Crossing accessions belonging to different clusters would maximize opportunities for transgressive segregation because of the higher probability that unrelated genotypes will contribute unique desirable alleles at multiple loci (Peters *et al*. 1989; Beer *et al*. 1993). Thus, the grouping of accessions by multivariate methods in the present study will be of practical value to barnyard millet breeders in allowing them to choose elite accessions from different clusters as parental lines for crossing programmes.

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