Classificatory Analysis in Little Millet Germplasm Collections of Odisha

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Twenty two genotypes of little millet (*Panicum sumatrense* Roth.) were evaluated under 12 environments. Data on 14 metric traits including grain yield were analysed to classify the entries into different groups following four methods of multivariate analysis. The genotypes were grouped into different clusters following D^2 analysis, canonical analysis, metroglyph analysis and numerical classificatory analysis. Clustering pattern in different methods indicated that the test genotypes were grouped into three clusters following D^2 analysis as well as canonical analysis, while they were divided into five groups in metroglyph analysis. Following the UPGMA method of numerical taxonomic approach, the genotypes were grouped into four, five and eight clusters at 75%, 80% and 85% phenon levels, respectively. The study of clusters formed by different methods indicated that the metroglyph analysis was easy and simple, and useful method of initial grouping for large number of collections. But the numerical taxonomic approach for classification of the biological populations is more potent in comparison to other methods to distinctly discriminate the genotypes for their use in recombination breeding.

Key Words: Canonical, D^2 static, Little millet, Metroglyph and numerical classificatory analysis, Multivariate analysis, *Panicum sumatrense*

Introduction

Genetic diversity in germplasm lines is generally considered as an important criterion in deciding appropriate plant breeding methods for crop improvement. Precise information on the nature and magnitude of genetic divergence in the population helps the plant breeder in choosing the diverse parents for purposeful hybridization (Arunachalam, 1981; Samsuddin, 1985). The importance of cluster analysis to determine the extent of variability was reported earlier (Mahalanobis, 1936). D^2 statistics has been utilized extensively for estimating genetic divergence in a number of crop plants with diverse breeding systems (Murty and Arunachalam, 1966; Bhatt, 1970). Although D^2 statistics is a quantitative measure of genetic divergence, the clustering pattern of the genotypes is arbitrary. The classification using generalized distance is workable when the number of entries is not very large. While classifying large number of germplasm collections in rice, Vairavan *et al.* (1973) used canonical analysis for initial grouping. But simple two-dimensional representation of multidimensional disposition of varieties cannot be as exact as the Tocher's method of grouping which scans the full multidimensional space, even when the two canonical vectors account for high proportion of variation (Arunachalam, 1981). On the other hand, metroglyph analysis has been used in many crop plants as a method for initial grouping (Anderson, 1957). In the numerical classificatory analysis, the general similarity coefficient of Gower (1971) has been used for clustering of populations at appropriate phenon level (Sneath and Sokal, 1973). An attempt has been made in the present study to classify 17 little millet germplasm collections from Odisha along with five check varieties. The classification of germplasm into different clusters following these methods was compared for determination of genetic closeness or divergence among the germplasm. The efficient method(s) would be preferred by plant breeders for an effective classificatory analysis of various crops.

Materials and Methods

The investigation was carried out at the Central Research Station, Orissa University of Agriculture & Technology (OUAT), Bhubaneswar, during the period from *kharif* 2006 to *kharif* 2007 under 12 different environmental conditions generated through different dates of sowing. The test genotypes consisting of 17 local germplasm collected from nine different districts of Odisha were evaluated along with 2 varieties of OUAT, Bhubaneswar and three varieties of Tamil Nadu Agricultural University

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(TNAU), Coimbatore as checks. The genotypes were sown in a Randomized Complete Block Design (RCBD) with three replications and observations were recorded for grain yield and 13 component characters. D^2 analysis and clustering by Tocher's method were done following Rao (1952). Canonical analysis was carried out according to Anderson (1957). For grouping in metroglyph analysis, a method was devised to delineate the performance of the genotypes into three classes in respect of yield and ten other component characters. The grand mean (M) and standard deviation (SD) were calculated for each character. The performance of the entries in respect of the eleven characters was ranked as high with mean values more than (M + 0.7SD), low with mean values less than (M – 0.7SD) and medium with mean values of (M ± 0.7SD). Accordingly, the genotypes were grouped into three classes i.e. low, medium and high for each of the eleven characters and numerically scored on three point scale as 1, 2 and 3 respectively. In the numerical classificatory analysis, the general similarity coefficient (S_G) of Gower (1971) was used as a measure of resemblance between different operational taxonomic units or OTUs (entries included in the investigation). The S_G values were calculated following Sneath and Sokal (1973). Basing on the matrix of the S_G values, phenongram (dendrogram) was constructed using UPGMA (un-weighted pair-group method using arithmatic average) technique in one of the SAHN (sequential, agglomerative, hierarchic, non-overlapping) clustering methods. Finally, the clusters were identified at appropriate phenon levels.

Table 1. Analysis of variance for 14 characters of 22 genotypes of little millet pooled over 12 environments

Character	Mean square				
	Reps/Env. (24)@	Genotype (21)	Environ-ment (11)	G X E (231)	Pooled error (504)
Grain yield (GY)	3.0	98.19**	2731.49**	20.51**	2.20
Days to heading (DH)	2.6	128.80**	989.28**	12.08**	0.82
Plant height (PH)	2.8	660.65**	3828.32**	45.47**	4.43
Flag leaf length (FLL)	0.5	29.03**	270.68**	6.51**	0.66
Flag leaf area (FLA)	0.2	19.72**	170.48**	3.52**	0.24
Panicle length (PL)	0.3	62.78**	391.09**	6.75**	0.70
Panicle exsertion (PE)	0.1	8.76**	847.30**	3.11**	0.26
Panicle number (PN)	3.5	410.53**	6835.36**	141.95**	7.48
Panicle weight (PW)	0.002	0.05**	0.92**	0.01**	0.001
Panicle yield (PY)	3.8	153.01**	3765.77**	33.49**	2.92
Straw yield (SY)	1.4	301.29**	9817.65**	90.28**	4.11
Biological yield (BY)	5.5	757.60**	18834.86**	156.46**	7.56
Harvest index (HI)	8.5	97.18**	4478.94**	36.20**	5.05
1000-grain weight (GW)	0.01	0.44**	2.09**	0.02**	0.0004

@Figures in parentheses indicate the degrees of freedom

Results and Discussion

Pooled analysis of variance over environments showed highly significant differences among the test genotypes in respect of all 14 characters (Table 1). The significant differences due to environments and differential response of the genotypes to changing environments as revealed from the significance of interaction components warranted grouping of the genotypes to identify the genetically diverse ones to ensure success in recombination breeding. The bias in clustering of genotypes in different environments, presumably due to differential response of different characters, could be eliminated by pooled analysis.

In the multivariate analysis, 14 characters contributed differently to the total D^2 for each pair of varieties. The major contributors to genetic diversity were 1000-grain weight (33 %) and days to heading (17%), accounting for 50% of the total divergence followed by plant height (15%). Contribution of days to flowering and 1000-grain weight to total divergence has also been reported in rice, common millet and little millet (Mahapatra *et al.,* 1995, Reddy *et al.,* 1996; Arunachalam *et al.,* 2005). Following the Tocher's method, the twenty-two entries were grouped into three clusters and the clustering was in broad agreement with the groupings obtained by using the first two canonical vectors. Cluster I comprised of 20 genotypes which included four released varieties namely Kolab and Sabar (released from OUAT, Bhubaneswar) and PRC3 and CO 2 (released from TNAU, Coimbatore). Cluster II consisted of KCM 309, one local germplasm of Kalahandi district of Odisha, while cluster III contained check variety TNAU 98 (released

from TNAU, Coimbatore). The most divergent clusters were cluster II and III followed by clusters I and III having average inter-cluster distance (D^2 value) of 67.7 and 36.5, respectively. Hence, hybridization between genotypes in diversed clusters is likely to yield better recombinants in segregating population.

In the metroglyph analysis, computation of scoring index of the genotypes in respect of the characters revealed that the genotype TNAU 98 had the highest score, while KCM 594 and RCM 4 had the lowest score (Table 2). Basing on the scoring index, the genotypes were grouped into five clusters:

Cluster I:	KCM 84, KCM 103, KCM 121, KCM 153, KCM 309, KCM 404, KCM 405, RCM 7, RCM 10, RCM 16, RCM 20, RCM 22, Kolab, PRC 3
Cluster II:	KCM 42, KCM 102D, Sabar, CO 2
Cluster III:	KCM 594, RCM 4
Cluster IV:	RCM 17
Cluster V	TNAU 98

In this classification, the single multivariety cluster of D^2 analysis was observed to form three multivariety clusters indicating the subtle differences among the genotypes in the group. Hence, crosses between such genotypes in a cluster (cluster I of D^2 analysis) will provide scope for obtaining transgressive segregants in recombination breeding programme.

In the numerical taxonomic approach, all the 14 characters were used to calculate similarity coefficients (S_G) and the dendrogram prepared on the basis of similarity coefficients showed the different clusters at various phenon levels (Table 3). The genotypes could be broadly classified into four clusters both at 70 % and 75 % phenon level, *viz.* cluster I comprising 18 genotypes, cluster II consisting of one, cluster III with two genotypes and cluster IV having one genotype. While increasing the phenon level to 80 % and 85 %, the multivariate cluster I was further dissociated into two and five sub-clusters, respectively. The 18 genotypes in cluster I were grouped into two sub-clusters, *i.e.* IA and IB comprising of fourteen and four genotypes, respectively at 80 per cent phenon level. When the phenon level was increased up to 85 per cent, the sub-clusters IA and IB were further sub-divided into three and two genotypic constellations, respectively. Thus sub-cluster IA was further divided into IA_1, IA_2 and IA_3 with 11, 2 and 1 genotypes, respectively. Similarly, sub-cluster IB was dissociated into IB_1 and IB_2, each with two genotypes. Through the numerical taxonomic approach, it was possible to discern the subtle genotypic differences between the test entries grouped into different clusters and/or subclusters at different phenon levels. This subtle

Table 2. Numerical score of mean performance of 22 genotypes of little millet for 11 characters pooled over 12 environments

Genotype	**GY	PH	FLA	PL	PE	PN	PW	BY	HI	DH	GW	Total Score
KCM 42	3	2	2	2	2	2	3	2	3	2	2	25
KCM 84	3	2	2	2	2	3	2	2	2	2	2	24
KCM102D	3	2	2	2	2	2	3	3	3	3	2	27
KCM 103	2	1	2	2	2	3	1	1	2	2	2	20
KCM 121	2	2	2	2	2	3	2	2	2	2	2	23
KCM 153	2	2	2	2	2	2	2	2	2	2	2	22
KCM 309	2	3	2	2	1	1	3	2	1	3	1	21
KCM 404	2	2	1	2	2	2	2	2	2	2	2	21
KCM 405	2	2	2	2	1	2	2	2	2	2	2	21
KCM 594	1	1	1	1	2	1	1	1	1	1	2	13
RCM 4	1	1	1	1	2	1	1	1	1	1	2	13
RCM 7	2	2	2	1	3	2	3	2	3	2	2	24
RCM 10	1	2	2	2	3	2	1	1	2	2	2	20
RCM 16	2	2	2	2	3	2	2	2	2	2	2	23
RCM 17	1	2	2	1	2	1	2	2	2	2	1	18
RCM 20	2	2	2	2	2	2	2	2	2	2	2	22
RCM 22	2	2	2	1	3	1	3	2	3	2	2	23
Kolab	2	2	2	2	1	3	1	2	1	2	2	20
Sabar	2	2	3	2	2	3	2	3	2	2	2	25
PRC 3	1	2	2	2	2	2	1	2	1	2	2	19
CO 2	3	2	2	2	2	3	2	3	2	2	2	25
TNAU 98	3	3	3	3	3	2	3	3	2	3	3	31
											Mean	**21.82**
											SD	**04.05**
											0.7SD	**02.84**

**N:B: Abbreviations for characters as in Table 1.

.Table 3. Composition of clusters /sub-clusters identified from the dendrogram at different phenon level

Number of clusters/sub clusters at different phenon level			Genotypes
75%	80%	85%	
I (18)*	I A (14)	IA_1	KCM 84, KCM 103, KCM 12, KCM 153, KCM 404, KCM 405, RCM 10, RCM 16, RCM 20, Kolab, PRC 3
		IA_2	RCM 7, RCM 22
		IA_3	RCM 17
	I B (4)	IB_1	KCM 42, KCM 102D
		IB_2	Sabar , CO 2
II (1)	II (1)		KCM 309
III (2)	III (2)		KCM 594, RCM 4
IV (1)	IV (1)		TNAU 98

* Figures in parentheses indicate number of genotypes in the respective clusters

genetic differences among the test entries gives scope for selection of desirable types in recombination breeding programme.

A comparison of different clustering patterns showed that the genotypes were grouped into three clusters in D^2 analysis, five in metroglyph analysis, and four, five and eight clusters in UPGMA method of numerical taxonomic approach at 75 %, 80 per cent and 85 % phenon levels, respectively. It is therefore evident that the metroglyph analysis seems to be easy and simple, and can be used for initial grouping when the number of collections is large. But the numerical taxonomic approach for classification of the biological populations into different groups is more potent to distinctly discriminate the genotypes for their use in recombination breeding.

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Table 3. Composition of major fruit volatiles in Russian olive variants

S. No.	Retention time (minute)	Relative proportion of each volatiles (% of total volatiles)				
		Bee	Balti	Ringmo	Marpo	Chapacha
1	2.70	–	–	1.57	1.40	–
2	4.04	–	2.18	–	–	–
3	4.39	30.06	20.01	27.88	23.64	47.31
4	5.52	23.30	24.18	27.64	25.13	23.57
5	6.03	1.55	2.37	7.96	0.90	2.49
6	6.87	6.26	7.25	2.33	4.31	2.89
7	9.94	18.66	23.09	15.33	24.51	8.04
8	10.33	2.45	2.87	1.64	1.72	0.89
9	15.74	1.78	0.94	1.00	2.46	4.15
10	17.28	0.28	3.27	4.64	3.69	4.44
11	others	15.6	13.84	10.01	12.24	6.22

"–" indicates absence.

by genotype, which provides a recognition tool for chemotaxonomy (Laitinen *et al*., 2000).

In the present study also, the chromatographic data were found to be of taxonomic importance. These data indicated that the five fruit morphotypes of Russian olive were distinct from each other in the phytochemical compositions of their fruits and flowers. This is the first report producing chromatographic profile of fruit and flower extracts and fruit volatiles of Russian olive in which variant-specific chemical pattern was observed. Results of TLC and HPLC indicated the presence of stigmasterol only in the fruits of *bee* variant. Presence of stigmasterol in Russian olive fruit has not been reported previously, though other sterol, β-sitosterol has been reported in this species in earlier studies (Goncharova and Glushenkova, 1990; Goncharova *et al*., 1993).

These results thus confirm diversity based upon phytochemical profiling present among the variants of the species found in Ladakh, corroborating with existing ethnobotanical information and morphometric data. These profiles can be used as fingerprints for the identification of these varieties or to distinguish this species from other species.

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