RESEARCH ARTICLE
J. Crop Sci. Biotech. 11 (1) : 63 ~ 68

Genetic Divergence Analysis among Micromutant Lines in Finger Millet (*Eleusine coracana* G.)

Kumuda Chandra Muduli[1*], Rama Chandra Misra[1]

[1]*Department of Plant Breeding and Genetics, Orissa University of Agriculture and Technology, Bhubaneswar-751003, Orissa, India*

Abstract

The induced genetic divergence was estimated in 44 mutant lines of finger millet variety GPU 26, developed by single and combination treatments with gamma rays, EMS and NG using three multivariate analyses. The mutant lines were grouped into eight genetically diverse clusters by multivariate D2 and canonical analyses and 11 clusters by dendrogram grouping through Gower's similarity coefficient. The clustering pattern in these three methods was almost similar. Twelve mutant lines in D2 and 13 in the dendrogram grouping method were grouped in the parental cluster (Cluster I) indicating that they did not possess enough divergence from the parent to be classified as micromutant lines. However a large proportion of mutant lines showed divergence from the parent variety and also among themselves. No definite relationship of mutagenic origin and clustering of mutant lines were observed. The mutant lines developed from the same mutagenic treatments often grouped into different clusters indicating that each mutagenic treatment was effective in inducing diverse types of changes in the nine traits studied. The hybridization program between the divergent mutant lines GE 2-2 or GE 3-4 with GG 3-1 is expected to give promising and desirable segregants in subsequent generations. Traits such as days to 50% flowering and days to maturity had major contributions to the induced genetic divergence.

Key words: finger millet, genetic divergence, micromutant lines, yield components

Introduction

The primary objective of most mutation studies is to induce additional variability for crop improvement. Thus it would be of interest to ascertain how the different micromutant lines developed from the same parental variety differ among themselves and also from the parent. Univariate analysis would not be adequate to distinguish the micromutants with minor differences in many of the quantitative traits. Alternatively, multivariate analysis which takes into consideration several quantitative traits simultaneously would be a dependable method in determining stable differences among the micromutant lines. Use of multivariate D^2 and canonical analysis for identification and classification of micromutants has earlier been reported in green gram (Mohapatra et al. 1987) and finger millet (Giri 2002). Hierarchical cluster analysis using similarity coefficients (dendrogram grouping) was also used for classifying genotypes of wheat (Prakash and Joshi 2003) and forage sorghum (Yadav et al. 2004). All three methods of classification have also been used to study genetic diversity among dahlia varieties (Mishra et al. 2001). In the present study, the three different methods of multivariate analysis were used for identification and classification of micromutant lines in finger millet.

* **To whom correspondence should be addressed**
Kumuda Chandra Muduli
E-mail: muduli4@yahoo.co.in

Materials and Methods

The finger millet variety GPU 26 was used to induce mutation with gamma rays, ethyl methane sulphonate (EMS), and nitroso guanidine (NG). Nine single and two combination mutagenic treatments were taken. The single treatments were 15, 30, and 45 kr gamma rays (coded as G1, G2, and G3); 0.15, 0.30, and 0.45% EMS (E1, E2, and E3) and 0.015, 0.030, and 0.045% NG (N1, N2, and N3). The two combination treatments were 30 kr gamma rays + 0.30% EMS (GE2) and 30 kr gamma rays + 0.030% NG (GN2). The M_1 generation was bulk harvested and M_2 generation was grown. Ninety random, normal-looking plants were observed in each treatment and 16.7% plants were selected on the basis of higher yield in M_2 generation. Then 26.7% of M_3 progenies were selected on the basis of progeny yield and in all, forty-four micromutant lines were selected for M_4 studies. In the M_4 generation, these forty-four micromutant lines were grown along with the parent variety in randomized

block design with three replications during the wet season 2004. Twenty-one days-old seedlings were transplanted in three rows/entry and each row having thirty hills with single seedling/hill and 30 x 10 cm spacing. Observations on days to 50% flowering and maturity duration were recorded on plot basis. Plant height, tillers/plant, fingers/ear, finger length, 1000-grain weight, ear weight/plant and grain yield/plant were recorded on ten randomly selected plants from each entry. Multivariate analysis of genetic divergence among the micromutant lines was done using Mahalonobis's D^2-statistic, and canonical roots and clustering were done by Tocher's method (Rao 1952). For hierarchical cluster analysis, the general similarity coefficient of Gower (S_G) was used as a measure of resemblance between different operational taxonomic units or OTUs (entries in the study) and dendrogram was constructed based on S_G values using the unweighted pair group method using arithmetic average (UPGMA) technique (Sneath and Sokal 1973). Then the clusters were identified at 80 and 85% phenon levels.

Results

The analysis of variance revealed significant differences among the mutant lines for all the nine characters indicating that the mutagenic treatments were effective in inducing mutations in these polygenic traits and the treatments showed wide diversity among themselves.

The genetic divergence (D^2) among the 45 entries comprising the 44 micromutant lines and parent variety varied from 1.84 to 302.56 and 812 of the 990 D^2 estimates were significant indicating considerable divergence among the entries. The D^2 values of the mutant lines from the parent variety ranged from 1.84 to 182.66 and were high (more than 100) for the lines GGN 2-1 and GN 3-4. All the mutant lines along with the parent variety were grouped into eight genetic clusters by Tocher's method of grouping based on D^2 values (Table 1). Cluster I was the largest comprising the parent variety (GPU 26) and 12 mutant lines of which five were derived from gamma rays, two from EMS, four from NG and one from combination treatments. Cluster II was the second largest with 11 mutant lines of which one was from gamma rays, four from EMS, two from NG and four from combination treatments. Four mutant lines from EMS and three from NG treatments were included in Cluster III. The clusters IV to VIII included one to four mutant lines. It was observed that the mutants developed from gamma ray treatments were included in five clusters (I, II, IV, V, and VI) while those from EMS were in four clusters (I, II, III, and IV), and NG treatments in six different clusters (I, II III, V, VII, and VIII). Of the eight mutants from combination treatments, four were grouped in Cluster II, two in Cluster VI and one each in Cluster I and VII.

Canonical analysis was done for further confirmation of the clustering pattern of the mutant lines in Tocher's method using D^2 values. The first two canonical roots (Z_1 and Z_2) accounted for 58.3 and 11.0% of the diversity among the mutant lines and the grouping by this method was in broad conformity with the grouping by Tocher's method using D^2 values as evident from the scatter of points representing the mutant lines on the Z_1-Z_2 graph (Fig. 1).

The grouping by Gower's similarity coefficient (dendrogram) at 80% phenon level revealed that all the forty-four mutant lines were broadly grouped into 11 clusters (Fig. 2, Table 2). Cluster I was the largest cluster comprising the parent and 13 mutant lines (five from gamma rays, three from EMS, four from NG,

Table 1. Clustering pattern of 44 mutant lines of finger millet based on D^2 values.

Cluster no.	Number of cultures	Name of the culture (with entry number)
I	13	GG 1-1 (1), GG 1-4 (4), GG 2-2 (6), GG 2-4 (8), GG 3-3 (11), GE 1-3 (15), GE 2-3 (19), GN 1-1 (25), GN 2-3 (31), GN 2-4 (32), GN 3-1 (33), GGN2-1 (41), GPU 26 parent (45)
II	11	GG 3-4 (12), GE 1-1 (13), GE 1-2 (14), GE 3-1 (21), GE 3-3 (23), GN 1-4 (28), GN 3-2 (34), GGE2-2 (38), GGE2-4 (40), GGN2-2 (42), GGN2-3 (43)
III	7	GE 1-4 (16), GE 2-2 (18), GE 2-4 (20), GE 3-4 (24), GN 1-3 (27), GN 2-1 (29), GN 2-2 (30)
IV	3	GG 2-1 (5), GE 2-1 (17), GE 3-2 (22)
V	4	GG 1-3 (3), GG 2-3 (7), GG 3-1 (9), GN 1-2 (26)
VI	4	GG 1-2 (2), GG 3-2 (10), GGE 2-3 (39), GGN 2-3 (44)
VII	2	GN 3-4 (36), GGE 2-1 (37)
VIII	1	GN 3-3 (35)

Table 2. Composition of clusters/sub-clusters identified from the dendrogram of mutant lines.

Cluster	No. of entries	Sub-cluster group	No. of entries	Name of mutant line (with entry number)
I	14	IA	5	GG 2-2 (6), GG 2-3 (7), GN 2-3 (31), GN 3-1 (33), Parent (45)
		IB	2	GG 2-4 (8), GE 1-3 (15)
		IC	7	GG 1-1 (1), GG 1-4 (4), GE 1-4 (16), GE 2-3 (19), GN 1-1 (25), GN 1-3 (27), GGN 2-1 (41)
II	1	-	-	GGN 2-3 (43)
III	5	IIIA	4	GG 3-4 (12), GE 3-3 (23), GGE 2-2 (38), GGE 2-4 (40)
		IIIB	1	GG 3-3 (11)
IV	1	-	-	GG 1-3 (3)
V	1	-	-	GG 3-2 (10)
VI	5	VIA	3	GG 1-2 (2), GE 1-1 (13), GE 3-1 (21)
		VIB	1	GN 2-4 (32)
		VIC	1	GG 3-1 (9)
VII	7	VIIA	3	GE 1-2 (14), GN 3-2 (34), GGN 2-2 (42)
		VIIB	2	GN 3-4 (36), GGN 2-4 (44)
		VIIC	1	GGE 2-3 (39)
		VIID	1	GN 3-3 (35)
VIII	1	-	-	GN 1-2 (26)
IX	5	IXA	4	GE 2-2 (18), GE 2-4 (20), GN 1-4 (28), GN2-1 (29)
		IXB	1	GE 3-4 (24)
X	4	XA	2	GE 2-1 (17), GE 3-2 (22)
		XB	1	GG 2-1 (5)
		XC	1	GN 2-2 (30)
XI	1	-	-	GGE 2-1 (37)

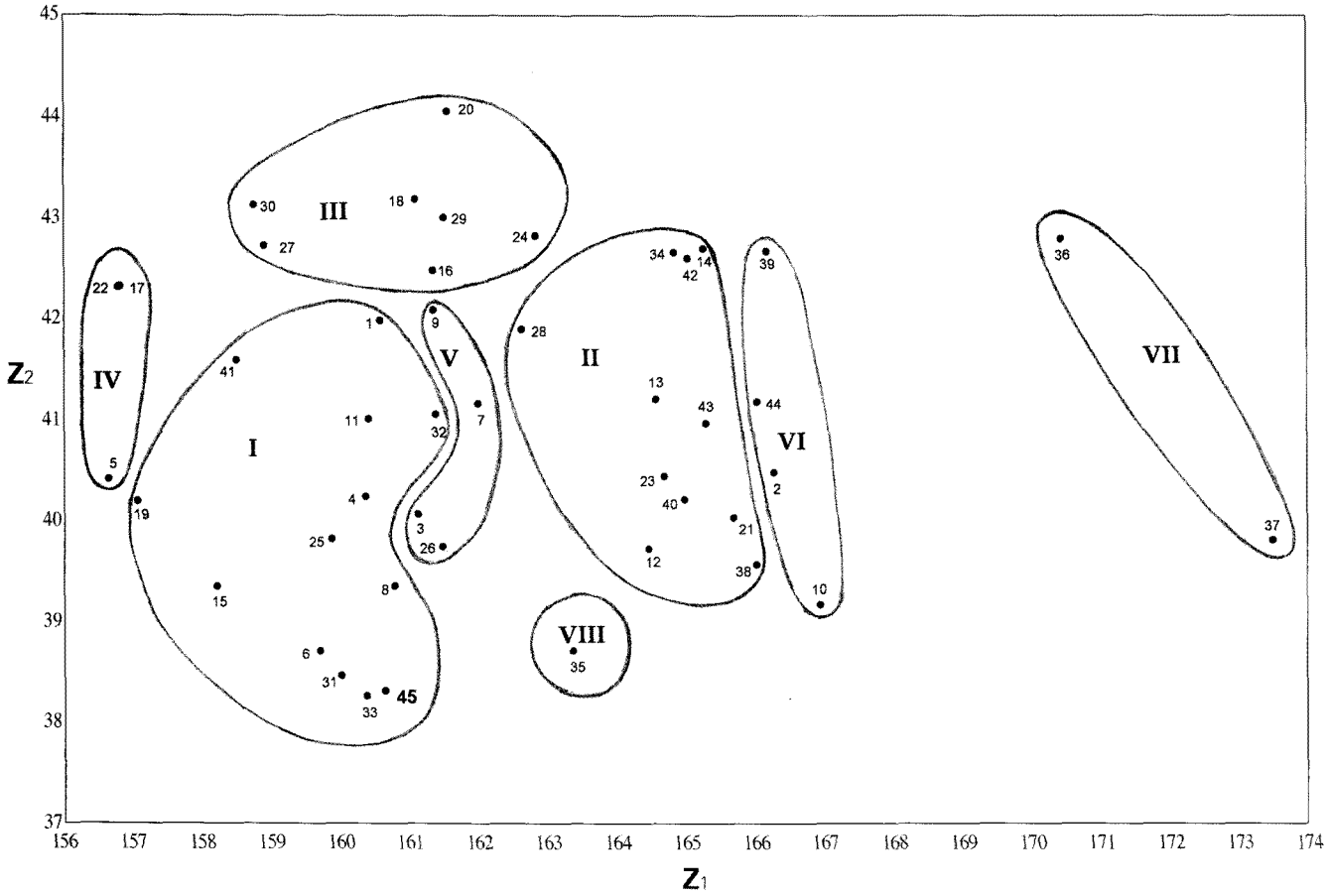

Fig. 1. Scatter of points representing the mutant lines in Z_1-Z_2 graph with D^2 clustering superimposed

and one from combination treatments). The remaining 31 lines were grouped into ten other clusters away from the parental cluster. Cluster VII included seven lines of which one was from EMS and three each from NG and combination treatments. Five mutant lines were grouped into each of Cluster III (two each from gamma ray and combination treatments, and one from EMS treatments), Cluster VI (two each from gamma ray and EMS, and one from NG treatments), and Cluster IX (three from EMS and two from NG treatments). Cluster X had four lines of which one each was from gamma rays and NG and two from EMS treatments. The clusters II, IV, V, VIII, and XI included one mutant line each. The mutant lines from gamma rays and EMS treatments were grouped into six different clusters, while those from NG and combination treatments in seven and four clusters, respectively. At 85% phenon level, Cluster I was further divisible into three sub-clusters (IA, IB, and IC) with the parent variety in IA, and Cluster VII into four sub-clusters. Cluster VI and X included three sub-clusters each while Clusters III and IX included two sub-clusters. The remaining five clusters were not further divisible into sub-clusters.

A comparison of clustering patterns in different methods showed that it was almost similar in all three methods. The D^2 analysis followed by Tocher's method of grouping and canonical analysis brought out eight clusters each, similar in their constitution. Clusters in D^2 analysis corresponds to the observed clusters in dendrogram to the extent of 60% i.e., 27 entries out of 45 remained in the same cluster. However, it was seen that ten mutant lines grouped with the parent variety in Cluster I both in D^2 and dendrogram grouping. The remaining 34 lines were grouped into other clusters away from the parental cluster in D^2 or dendrogram grouping. The clustering by D^2 analysis showed divergence among different groups of mutant lines. But clustering through dendrogram exhibited not only the dissimilarity between clusters but also the hierarchical classification within and among clusters.

The intra-cluster divergence by D^2 analysis was highest in Cluster VII (25.52) followed by Clusters III and VI (Table 3).

Table 3. Average intra (bold) and inter-cluster D^2 values.

Cluster no.	I	II	III	IV	V	VI	VII	VIII
I	**16.09**	39.44	25.06	28.67	29.32	60.38	165.38	33.14
II		**13.87**	32.81	81.38	36.89	23.19	68.78	26.58
III			**19.18**	34.48	34.34	52.23	142.40	41.92
IV				**12.74**	55.78	109.69	248.65	66.71
V					**14.18**	50.78	155.44	38.84
VI						**18.34**	66.67	38.97
VII							**25.52**	105.78
VIII								-

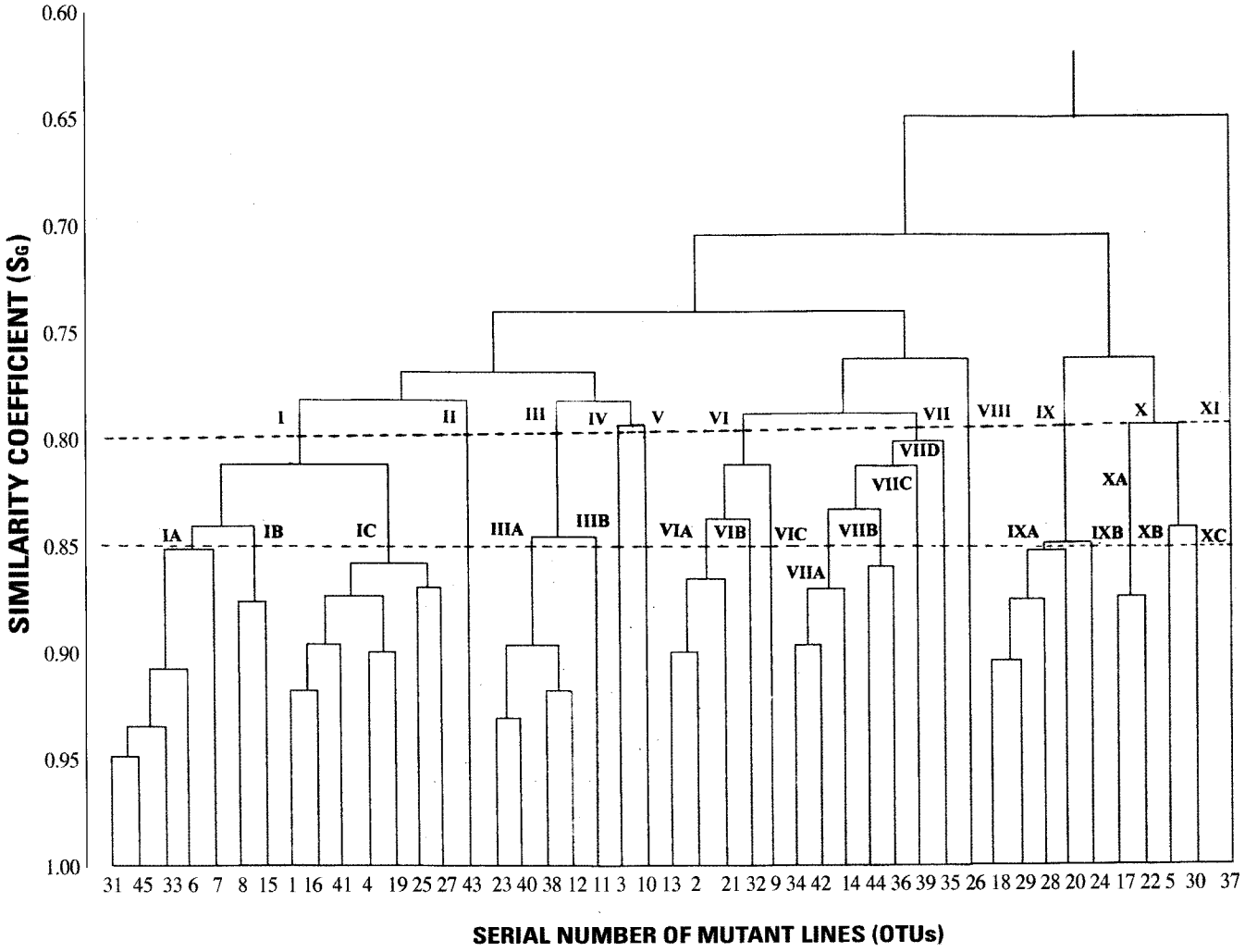

Fig. 2. Dendrogram of mutant lines based on Gower's similarity coefficient (S_G) values

The inter-cluster divergence was maximum (248.65) between Clusters IV and VII followed by Clusters I, VII, V, and VII, indicating they were diverse clusters. The Clusters II, III, IV, V, and VIII were relatively closer to the parental cluster (Cluster I) while the Cluster VII was far more diverse from the parental cluster.

The cluster mean for the nine characters showed that six clusters except Cluster VII recorded higher plant yield than the parental cluster (Table 4). The Cluster III with seven mutant lines recorded the highest grain yield/plant (12.32 g) and fingers/ear (8.07), short plant height, more tillers/plant, finger length, 1000-grain weight, and ear weight/plant but was slightly late in flowering and maturity duration. Cluster IV with three mutant lines (GG 2-1, GE 2-1, and GE 3-2) had better grain yield, short height, highest tillers/plant, and more fingers/ear and ear weight/plant with the earliest flowering and maturity. The other five clusters showed mean plant yield of 10.38 to 11.81 g and the mean values for different traits had wide varia-

Table 4. Mean of 9 characters in different clusters of mutant lines in D^2 analysis.

Cluster no.	Days to flowering	Days to maturity	Plant height (cm)	Tillers/ plant	Fingers/ ear	Finger length (cm)	1000-grain weight (g)	Ear weight/ plant (g)	Yield/ plant (g)
I	84.9	114.7	98.0	1.55	7.72	7.59	3.23	12.79	10.69
II	87.7	118.0	99.4	1.60	7.86	7.73	3.31	13.06	10.95
III	85.5	115.8	95.9	1.90	8.07	7.75	3.28	14.84	12.32
IV	83.0	113.0	92.8	1.93	7.77	7.45	3.21	14.54	12.18
V	85.5	114.9	104.2	1.47	7.54	7.99	3.50	13.25	11.15
VI	87.5	119.8	100.1	1.50	7.38	8.03	3.30	13.73	11.64
VII	92.2	122.3	99.1	1.67	7.70	7.70	3.28	12.59	10.38
VIII	87.0	117.3	100.7	1.67	7.67	7.22	3.35	14.85	11.81

Table 5. Trait contribution to the genetic divergence in D^2 analysis.

Traits	Average D^2	Percent of total D^2
Days to flowering	14.59	33.77
Days to maturity	9.99	23.11
Plant height (cm)	2.17	5.03
Tillers/plant	2.56	5.93
Fingers/ear	2.87	6.65
Finger length (cm)	3.52	8.15
1000-grain weight (g)	3.91	9.04
Ear weight/plant (g)	2.14	4.94
Yield/plant (g)	1.46	3.38

tion. Cluster V represented by four mutant lines (GG 1-3, GG 2-3, GG 3-1, and GN 1-2) had the highest 1000-grain weight and longer fingers. Close examination of the traits of mutant lines in Clusters III and V revealed that hybridization of GE 2-2 or GE 3-4 with GG 3-1 is expected to produce more transgressive segregants for yield.

Considering the contribution of different traits to genetic divergence among the mutant lines (Table 5) it was observed that days to 50% flowering and days to maturity were the major contributors to the genetic divergence indicating the induction of more heritable variation for these traits by the mutagenic treatments.

Discussion

The results of analysis of variance indicated significant differences among the micromutant lines in respect to all the characters suggesting that the different mutagenic treatments were effective in inducing micromutations in these polygenic characters. It was observed that vast majority of the D^2 estimates among the forty-five entries were significant indicating the effectiveness of mutagenic treatments in isolation of mutant lines with diverse changes in the traits studied.

The forty-four mutant lines and parent variety grouped into eight different clusters in Tocher's method of grouping based on D^2 values. The scatter of points representing the mutant lines in Z_1-Z_2 graph using canonical analysis were in broad agreement with D^2 grouping. Similarity coefficient (dendrogram) grouping at 80% phenon level produced 11 clusters. However this grouping showed a hierarchical representation of the clusters and each cluster could be further divided into sub-groups. From these three methods of grouping, it was quite evident that many of the mutant lines derived from the same parental variety showed genetic diversity/dissimilarity from the parent and also among themselves. Similar results have been reported earlier (Giri 2002; Parida 1997).

A close observation of the D^2 clustering pattern revealed that 12 mutant lines grouped with the parent variety in Cluster I. Similarly, on the basis of similarity coefficient, 13 mutant lines exhibited a similar trend. Thus, it appears that these mutant lines do not possess enough divergence/dissimilarity from the parent in the nine traits, to be classified as micromutants. On the basis of D^2 grouping, 32 mutant lines grouped into seven different genetic clusters while in similarity coefficient grouping, 31 lines grouped into ten different clusters away from the parental cluster. Thus, most of these mutant lines exhibited lot of divergence/dissimilarity in traits not only from the parent, but also among themselves to be classified into different clusters. Examination of the clustering pattern in both methods revealed that most clusters often included mutant lines derived from different mutagenic treatments. Conversely, mutant lines derived from same mutagenic treatments also grouped into different clusters. Therefore, no definite pattern of mutagenic origin and clustering of mutant lines were observed which was in agreement with the earlier findings (Misra 1995). Thus, it can be inferred that each mutagenic treatment was effective in inducing diverse types of changes for all the quantitative traits.

A large proportion of mutant lines showed divergence from the parent variety and also among themselves. Thus, some of these mutant lines with reasonably good yield and showing divergence between them in different traits, more particularly in productive traits, may be of breeding value to be used in hybridization programs. Examination of character mean of different D^2 clusters revealed wide differences in different traits. Six out of the eight clusters exhibited higher mean grain yield than the parental cluster (Cluster I). Cluster III represented by seven mutant lines (GE 1-4, GE 2-2, GE 2-4, GE 3-4, GN 1-3, GN 2-1, and GN 2-2), had the highest average plant yield and these lines showed very high increase in tillers/plant with moderate increase in fingers/ear than the parental cluster. Cluster IV (GG 2-1, GE 2-1, and GE 3-2) exhibited the second highest yield and conspicuous increase in tillers/plant. Cluster V with four lines (GG 1-3, GG 2-3, GG 3-1, and GN 1-2) had the highest 1000-grain weight and longer fingers. Close examination of the traits of mutant lines in Clusters III and V revealed that - hybridization of GE 2-2 or GE 3-4 with GG 3-1 is expected to produce more high yielding transgressive variants in subsequent generations, which would facilitate successful breeding of finger millet. Production of transgressive variants by hybridization of mutant lines has earlier been reported (Maluszynski et al. 1991; Micke 1976; Misra 1995). Moreover, the cluster means for different characters revealed the extent of diversity of groups of mutants from the parental cluster and also among themselves. Similar clusters with differential character means have also been reported in previous studies (Giri 2002; Parida 1997).

Days to 50% flowering and days to maturity were observed to be the major contributors to the genetic divergence. These traits may be useful for selection of more diverse mutant parents. The results are in broad agreement with the observations of some previous workers (Giri 2002; Panigrahi 1991; Parida 1997).

In the present investigation, it is suggested that grouping by D^2, canonical and similarity coefficient analyses can be used for identification and classification of micromutant lines of the same parental origin. Also, the hybridization programs between the divergent mutant lines GE 2-2 or GE 3-4 with GG 3-1 is expected to give promising and desirable segregants in subsequent generations.

Acknowledgements

The research facility provided by Dr. Laxmidhar Mishra, Professor and Head, Department of Plant Breeding and Genetics, Orissa University of Agriculture and Technology, Bhubaneswar to Dr. Kumuda Chandra Muduli is gratefully acknowledged.

References

Giri AK. 2002. Mutational improvement of ragi: Genetic variability among some mutant lines. M Sc. (Ag.) thesis (unpublished), Orissa Univ. of Agric. and Technol., Bhubaneswar, India

Maluszynski M, Sigurbjornsson B, Amono E, Sitch L, Kamara O. 1991. Mutation varieties-data Bank, FAO / IAEA Data Base. Mut. Breed. Newsl. 38: 16-49

Micke A. 1976. Introduction. Induced mutations in cross breeding. Proc. Adv. Group. IAEA, Vienna, pp 1-4

Mishra M, Mahapatra KC, Mohanty CR. 2001. Classificatory analysis of genetic diversity in dahlia. Indian J. Genet. 61: 187-189

Misra RC. 1995. Multivariate analysis of genetic divergence in micromutants developed from blackgram variety T9. Environ. Ecol. 13: 508-512

Mohapatra BK, Misra RC, Baisakh B. 1987. Genetic divergence among mutant lines of green gram. J. Orissa Bot. Soc. 9: 11-16

Panigrahi JC. 1991. Genetic divergence and index selection among the mutant lines of a brown ragi cultivar. M Sc. (Ag) thesis, Orissa Univ. of Agric. and Technol., Bhubaneswar, India

Parida D. 1997. Mutational improvement of ragi: Comparative spectrum and frequency of induced genetic variability. Ph D. thesis, Orissa Univ. of Agric. and Technol., Bhubaneswar, India

Prakash V, Joshi P. 2003. Genetic analysis of yield and its components in different environments in wheat (*Triticum aestivum L.*). Indian J. Genet. 63: 341-345

Rao CR. 1952. Advanced statistical methods in biometrical research. John Wiley and Sons Inc., New York

Sneath PHA, Sokal RR. 1973. Numerical Taxonomy. The Principles and practice of numerical classification. W H Freeman and Company, San Francisco, New York

Yadav R, Grewal PS, Pahuja SK. 2004. Multivariate analysis in forage sorghum (*Sorghum bicolor* L.). IndianJ. Genet. 64: 39-45