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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(7): 3611-3615

www.thepharmajournal.com
Received: 03-05-2023
Accepted: 07-06-2023

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Mahalanobis D^2 statistical technique for genetic diversity assessment in the Gamma radiated mutant population of kodomillet (*Paspalum scrobiculatum* L.)

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Abstract
The present study on induced mutations in kodomillet (*Paspalum scrobiculatum* L.) was carried out at the Instructional cum Research Farm of S.G. College of Agriculture and Research Station Kumhrawand, Jagdalpur, Chhattisgarh. Induced genetic divergence was estimated in M_1 mutant lines of kodomillet var. Indira kodo-1, developed by gamma rays using multivariate analysis using Mahalanobis' (1936). 102 lines of kodomillet grouped into five clusters based on D^2 analysis. The cluster III was largest with 27 lines followed by cluster V with 22 lines, cluster I and cluster IV having 19 lines, but cluster II was lowest 15 lines. The maximum intra-cluster distance was found in cluster I indicating difference in mutant lines within cluster. The highest inter-cluster distance was observed between clusters cluster IV and cluster II followed by cluster IV and cluster III showing high degree of genetic diversity indicating that genetic makeup of lines falling in this cluster may be entirely different from one another. Among the traits under studied, grain per panicle in mutated population contributed maximum toward divergence followed by seed weight per panicle in mutated population. In present study of D^2 analysis suggested that mutant lines belonging to the diverse clusters could be used in hybridization programmes to enhance the productivity of kodomillet.

Keywords: Kodomillet, *Paspalum scrobiculatum* L., induced mutation, genetic divergence

Introduction
Kodomillet (*Paspalum scrobiculatum* L.), a member of the family Poaceae, having chromosome no. 2n=2x=40 is a highly drought resistant crop and coarsest of all food grains was domesticated in India some 3,000 years ago (Malleshi and Hadimani, 1994) [12] and is cultivated as agricultural crop in parts of Madhya Pradesh, Maharashtra, Uttar Pradesh, Gujarat, Rajasthan and Tamil Nadu (De Wet *et al.* 1983) [4]. The creation and management of genetic variability becomes central base to crop breeding in any crop and more so in crops like Kodomillet, in which the available genetic variability is very limited owing to complete self-pollination in this crop due to its cleistogamous nature (Harinarayana, 1989) [6]. It is self-pollinating, florets generally remain closed during the flowering period. The grain occurs in a hard husk, making de-branning difficult (Kajuna, 2001) [7].

In India, millets are cultivated in an area of 12.45 million hectares, producing 15.53 million tonnes with a yield of 1247 kg/ha (Anonymous 2022) [1-2]. Chhattisgarh occupied 248.5 thousand hectares of land (which is 21.18% of India's 1173.5 thousand ha) under small millets with total area of 79.12 thousand hectare, the total production was 34.39 thousand tons and the total productivity was 435 kg/ha (Anonymous 2022) [1-2]. Kodomillet is a nutritious grain and a good substitute to rice or wheat. The protein, fiber and mineral content are much higher than the major cereals like rice. The major protein fraction in kodomillet is glutelin (Sudharshana *et al.* 1988) [21]. Kodomillet is an excellent source of fiber (9%), kodomillet contains 66.6 g of carbohydrates and 353 kcal per 100 g of grain, comparable to other millets. It also contains 1.4% fat and 2.6% minerals. The iron content in kodomillet ranges from 25.86ppm to 39.60 ppm (Chandel *et al.* 2014) [3]. With other food grains, the nutritive value of kodomillet protein could be improved by supplementation with legume protein (Deshpande *et al.* 2015) [5]. The induced mutations are of considerable value for comprehension, evaluation and accelerating the process of plant improvement. Crop plants can be created more diverse by inducing mutation through the application of either physical or chemical mutagens (Subramanian *et al.* 2011) [20].

Gamma rays are electromagnetic radiation that initiates and inhibit the growth of the plant. To produce the desired variation in plants, it is important to optimize the gamma irradiation dose (Patel *et al.* 2017) [16]. D^2 analysis was generally used by many researchers in order to find more divergent lines that can be used to make hybrids. Muduli and Misra (2008) [13] have done genetic divergence analysis in micro-mutant lines in finger millet and found divergent mutant lines. Similarly, Patel *et al.* (2019) [17]; Suryanarayana *et al*. (2019) [23]; Keerthana and Chitra (2020) [8]; Vasisth *et al*. (2022) [25] also conducted D^2analysis in finger millet and got divergent genotypes. In the present study, D^2 analysis was used to identify divergent mutant line from M_1 mutant lines of kodomillet.

Materials and Method

The present study on induced mutations in kodomillet was carried out at the Research cum Instructional Farm of S.G. College of Agriculture and Research Station, Jagdalpur, Bastar (C.G.), which was located at 19°5'35" N and 81°57'37" E, at an altitude of 552 meters in Bastar plateau above the main sea level. The experiment was conducted in two step, experiment-01 and experiment-02. For the experiment-01, healthy and dry seed of kodomillet were collected and packed in paper bags each of 12 sets were gamma radiated using Cobalt-60 or ^{60}Co (because of the relative long half-life, cheapness and availability of cobalt-60) source at Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre (BARC), Mumbai (MH). Seed were treated with different doses *viz.* 100 Gy, 200 Gy, 300 Gy, 400 Gy, 500 Gy, 600 Gy, 700 Gy, 800 Gy, 900 Gy, 1000 Gy, 1100 Gy and 1200 Gy, with irradiated seed serving as a control. The gamma ray treated seed of 100 each in each treatment were placed in aluminum tray for determination of LD_{50} dose and GR_{50} dose *in vitro* condition compared with the control. LD_{50} and GR_{50} were observed based on probit analysis or regression line.

For the experiment-02, dried seed were exposed to gamma radiation from Cobalt-60 at the BARC, Mumbai according to the LD_{50} dose and GR_{50} of experiment-01. The experiment was laid out as an Augmented Block Design with 10 blocks in agriculture field. Seeds were sown in each block in the prepared field condition. At harvest stage, irregular plants were selected for determining the genetic divergence in plant. At the harvest stage, plant height, flag leaf length, flag leaf width, panicle length, grain per panicle, seed weight per panicle and test weight were all recorded on randomly selected plants for each line in every block. Estimation of genetic divergence was done by multivariate analysis using Mahalanobis' (1936) [10]. D2 statistic calculated as described by Rao (1952) [18]. Contribution of each trait to the divergence, intra and inter cluster distance and cluster means were estimated in the present study.

Results and Discussion

Genetic diversity studies give basic knowledge about the genetic properties of genotypes, which are used to develop breeding strategies for crop improvement. These investigations are also helpful in understanding the nature and extent of diversity that can be linked to a variety of factors, such as crop sensitivity to the environment and genetic divergence. The Mahalanobis D^2 statistics approach helps in quantifying the divergence between two populations that can be used as an index for choosing parents of different origins while clustering lines using Rao's Tocher approach. Mahalnobis' (1936) [10] concept of D^2 statistics is a useful tool for plant breeders to classify genotypes into discrete groups based on genetic divergence. The main concept behind cluster formation is to find out intra- and inter-cluster distances. The index is applied to choose parents with diverse backgrounds. For 100 different lines of kodomillet, genetic divergence was estimated. These lines were grouped, by following Mahalanobis's D^2 as described by Rao (1952) [18].

Grouping of lines into clusters

The 102 kodomillet lines examined in this experiment is grouped into 5 clusters namely Cluster I, Cluster II, Cluster III, Cluster IV and Cluster V. The clusters along with lines included in them are presented. The cluster III was largest with 27 lines followed by cluster V with 22 lines, cluster I and cluster IV having 19 lines, but cluster II was lowest 15 lines. An indicator of the relative genetic closeness of lines is clustering. The genotypes present in same cluster are considered to be more closely related by their origin, ancestry and genetic make-up, compared to genotypes of other cluster. Clustering is crucial in crop improvement, for getting diverse parents in combination and transgressive breeding, and it is advised that parents should be chosen from different clusters. The fundamental concert behind formation of clusters is to get the intra and inter-cluster distances which is used as an indicator for selection of parents with diverse origin. The intra and inter-cluster values are means derived from D^2 values of cluster elements. It will be more accurate to cross genotypes put in clusters with large inter-cluster distances in order to obtain desired outcomes. It can be applied as a powerful screening method and for other breeding objectives. Based on D^2 values, (Kumari and Singh 2015) [9] defined six groups of finger millet genotypes and concluded that there was no formal association between geographic diversity and genetic diversity. Earlier it was also used as a viable criteria for effective selection and other breeding purposes (Suryanarayana *et al*., 2014 [22]; Kumari and Singh 2015) [9]. The cluster formation in kodomillet was reported by Nirubana *et al*., (2017) [14], Mahanthesha *et al*., (2017) [11] and Thakur *et al*., (2018) [24].

Intra and inter cluster distances

A basis for choosing genetically diverse parents from various clusters is provided by the cluster formation and measurement of intra and inter-cluster divergence. The statistical distance (D) is thought to be an indicator of genetic diversity. Intra and inter-cluster D^2 and D values were calculated using D^2 values from divergence analysis. The maximum intra-cluster distance was found in cluster I (2.118), followed by cluster IV (2.088), cluster V (1.985), cluster II (1.841) and minimum intra-cluster distance was observed for the cluster III (1.782). displays the intra-cluster distances among various clusters. This suggests that each of these clusters has lines with distinct genetic makeup. Minimum intra-cluster denotes relative genetic closeness of genotypes Suryanarayana *et al.*, (2014) [22]. Highest inter-cluster distance was founded between cluster IV and cluster II (3.513). Higher inter-cluster distance indicates that the lines present, have significant genetic distance among them. This was followed by cluster IV and cluster III (3.412) demonstrating that these clusters have a higher level of genetic divergence and that crossing genotypes from these clusters was resulted in higher level of heterotic

expression and a larger range of diversity in subsequent segregating populations Suryanarayana *et al.*, 2014) [22]. Simultaneously, the minimum D^2 value was found between cluster III and cluster I (2.155) indicating that genotypes of these two clusters have lack of genetic diversity. Increasing parental distance implies a greater number of contrasting alleles at the desired loci, and then to the extent that these loci recombine in the F2, F3 and later generation will determine the outcome of breeding programmes and importance of breeding material developed. (Wolie and Belete 2013) [26] revealed highest average inter-cluster D^2 in their experiment, indicating more genetic divergence among these clusters and crossing genotypes from these two clusters produce s individuals exhibiting greater Heterosis. The inter-cluster distance was larger than the intra-cluster distance, indicating broader genetic diversity among genotypes of different clusters, to the character considered. Therefore, hybridization across genotypes throughout the clusters may be used to produce combinations with strong heterotic response and improved recombinants. Low intra-cluster distance values were a sign of little genetic diversity within a cluster.

Cluster means for different quantitative characters

The cluster means for all 7 characters are presented in appreciable differences between clusters were observed for the characters studied which included plant height at maturity, flag leaf length, flag leaf width, panicle length, grain per panicle, grain weight per panicle and test weight. Cluster I included 19 genotypes *viz.* BK2022-15, BK2022-22, BK2022-25, BK2022-27, BK2022-32, BK2022-36, BK2022-41, BK2022-57, BK2022-66, BK2022-69, BK2022-70, BK2022-73, BK2022-81, BK2022-89, BK2022-91, BK2022-92, BK2022-97, Chhattisgarh kodo-2 and Indira kodo-1. None of the character in Cluster I had the high mean value. The cluster mean value was medium for the characters like flag leaf length (20.76), panicle length (5.67), grain per panicle (130.31), test weight (4.12) and grain weight per panicle (0.52). Some characters like plant height (80.14) and flag leaf width (0.82), demonstrated low cluster mean value. In case of cluster II, it contains 15 genotypes *viz.* BK2022-10, BK2022-14, BK2022-24, BK2022-28, BK2022-33, BK2022-34, BK2022-47, BK2022-52, BK2022-53, BK2022-56, BK2022-62, BK2022-64, BK2022-71, BK2022-93 and BK2022-94. With only one character came under high cluster mean value i.e. for plant height (89.56), The cluster mean value is medium for the characters like flag leaf width (0.84), grain per panicle (106.53), test weight (4.31) and grain weight per panicle (0.45), whereas some characters like flag leaf length (18.95) and panicle length (5.23) exhibited low cluster mean value.

Cluster III contains of 27 genotypes *viz.* BK2022-13, BK2022-16, BK2022-19, BK2022-20, BK2022-21, BK2022-26, BK2022-31, BK2022-44, BK2022-45, BK2022-46, BK2022-48, BK2022-50, BK2022-54, BK2022-58, BK2022-59, BK2022-60, BK2022-65, BK2022-72, BK2022-74, BK2022-75, BK2022-77, BK2022-84, BK2022-86, BK2022-90, BK2022-95, BK2022-98 and BK2022-100. None of the character in Cluster III had the high mean value. The medium mean cluster value was recorded for the characters like plant height (86.29), flag leaf length (22.65), flag leaf width (0.83) and panicle length (5.86) whereas some characters like grain per panicle (74.41), test weight (4.10) and grain weight per panicle (0.31) exhibited low cluster mean value. Cluster IV contains of 19 genotypes *viz.* BK2022-1, BK2022-2, BK2022-3, BK2022-4, BK2022-5, BK2022-8, BK2022-9, BK2022-11, BK2022-12, BK2022-23, BK2022-29, BK2022-30, BK2022-43, BK2022-63, BK2022-68, BK2022-76, BK2022-80, BK2022-85 and BK2022-99. The cluster mean value was high for the characters like flag leaf length (23.90), grain per panicle (106.53), test weight (5.75) and grain weight per panicle (0.93). Some characters like plant height (87.71), flag leaf width (0.83) and panicle length (5.88) exhibited medium cluster mean value. None of the character in Cluster IV had the low mean value.

Cluster V included 22 genotypes *viz.* BK2022-6, BK2022-7, BK2022-17, BK2022-18, BK2022-35, BK2022-37, BK2022-38, BK2022-39, BK2022-40, BK2022-42, BK2022-49, BK2022-51, BK2022-55, BK2022-61, BK2022-67, BK2022-78, BK2022-79, BK2022-82, BK2022-83, BK2022-87, BK2022-88 and BK2022-96. The high mean cluster value was recorded for the characters like plant height (87.97), flag leaf width (0.89) and panicle length (6.10) whereas some characters like grain per panicle (133.14), flag leaf length (23.73) test weight (4.70) and grain weight per panicle (0.62) exhibited medium cluster mean value. None of the character in Cluster V had the low mean value. Among the traits under studied, grain per panicle in mutated population contributed maximum toward divergence followed by seed weight per panicle in mutated population. It was noticed that lines with high cluster mean values for a particular character might be used in a breeding program for trait enhancement. Sao *et al.* (2016) [19] and Thakur *et al.* (2020) [24] studied intra and inter cluster divergence in kodomillet and found variability for most of the traits.

Table 1: Cluster classification of kodomillet mutant lines

Clusters	No of genotypes	Mutant lines
I	19	BK2022-15, BK2022-22, BK2022-25, BK2022-27, BK2022-32, BK2022-36, BK2022-41, BK2022-57, BK2022-66, BK2022-69, BK2022-70, BK2022-73, BK2022-81, BK2022-89, BK2022-91, BK2022-92, BK2022-97, Chhattisgarh kodo-2, Indira kodo-1
II	15	BK2022-10, BK2022-14, BK2022-24, BK2022-28, BK2022-33, BK2022-34, BK2022-47, BK2022-52, BK2022-53, BK2022-56, BK2022-62, BK2022-64, BK2022-71, BK2022-93, BK2022-94
III	27	BK2022-13, BK2022-16, BK2022-19, BK2022-20, BK2022-21, BK2022-26, BK2022-31, BK2022-44, BK2022-45, BK2022-46, BK2022-48, BK2022-50, BK2022-54, BK2022-58, BK2022-59, BK2022-60, BK2022-65, BK2022-72, BK2022-74, BK2022-75, BK2022-77, BK2022-84, BK2022-86, BK2022-90, BK2022-95, BK2022-98, BK2022-100
IV	19	BK2022-1, BK2022-2, BK2022-3, BK2022-4, BK2022-5, BK2022-8, BK2022-9, BK2022-11, BK2022-12, BK2022-23, BK2022-29, BK2022-30, BK2022-43, BK2022-63, BK2022-68, BK2022-76, BK2022-80, BK2022-85, BK2022-99,
V	22	BK2022-6, BK2022-7, BK2022-17, BK2022-18, BK2022-35, BK2022-37, BK2022-38, BK2022-39, BK2022-40, BK2022-42, BK2022-49, BK2022-51, BK2022-55, BK2022-61, BK2022-67, BK2022-78, BK2022-79, BK2022-82, BK2022-83, BK2022-87, BK2022-88, BK2022-96,

Table 2: Average intra and inter-cluster D^2 values in 5 clusters of mutant lines of kodomillet

Clusters	I	II	III	IV	V
I	2.118				
II	2.467	1.841			
III	2.155	2.286	1.782		
IV	3.096	3.513	3.412	2.088	
V	3.073	3.07	2.516	2.296	1.985

Table 3: Cluster means components of 5 clusters of kodomillet mutant lines

Cluster No.	Plant height	Flag leaf length	Flag leaf width	Panicle length	Grain per panicle	Test weight	Seed weight per panicle
I	80.14	20.76	0.82	5.67	130.31	4.12	0.52
II	89.56	18.95	0.84	5.23	106.53	4.31	0.45
III	86.29	22.65	0.83	5.86	74.41	4.10	0.31
IV	87.71	23.90	0.83	5.88	162.47	5.75	0.93
V	87.97	23.73	0.89	6.10	133.14	4.70	0.62

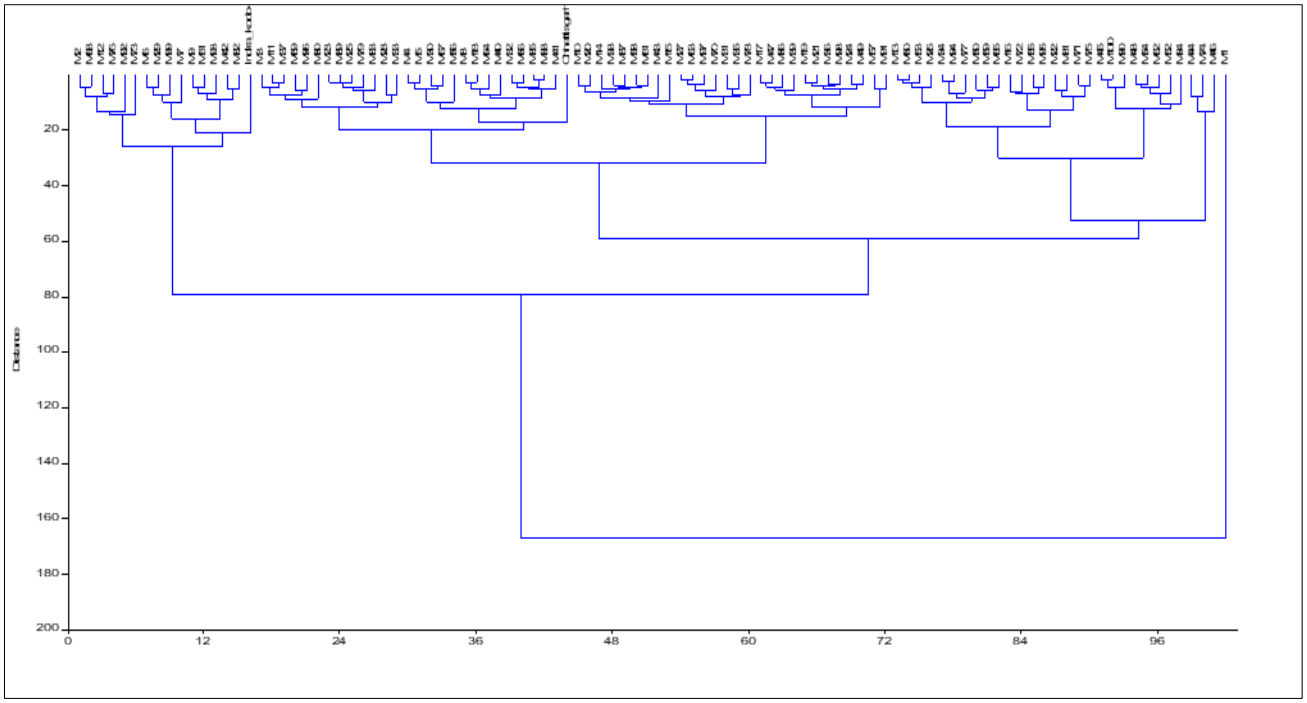

Fig 1: Dendrogram showing clustering pattern of 102 lines

Acknowledgement
The investigation was a part of M.Sc. thesis executed at S.G. College of Agriculture and Research Station, Jagdalpur, IGKV, Raipur, Chhattisgarh. The work was funded by ICAR-IIMR, Hyderabad and DRS, IGKV, Raipur. The authors duly acknowledge all the institutes for technical and financial support for research.

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