Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(6): 1373-1377
Received: 09-07-2020
Accepted: 15-10-2020

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Genetic divergence studies in kodo millet (*Paspalum scrobiculatum* L.)

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Abstract
The present study on "Genetic Divergence Studies in Kodo Millet (*Paspalum scrobiculatum* L.)" was carried out at Instructional cum Research Farm of S.G. College of Agriculture and Research Station Kumhrawand, Jagdalpur, Chhattisgarh. The 33 genotypes were grouped into 6 different clusters based on D^2 analysis. Cluster V was largest with 11 genotypes followed by cluster IV with 7genotypes, cluster II with 6 genotypes cluster III and cluster I both had 4 genotypes, cluster VI with 1 genotype. The maximum intra-cluster difference was found in cluster IV indicate that the genotype present, have considerable genetic distance among them. The maximum inter cluster difference was found between cluster I and cluster VI showing high degree of genetic diversity indicating that genetic makeup of genotypes falling in this cluster may be entirely different from one another. Among the trait under studied, days to maturity contributed maximum towards diversity.

Keywords: Kodo millet, *Paspalum scrobiculatum* L., Cluster analysis

Introduction
Kodo millet (*Paspalum scrobiculatum* L.) is a tropical small millet indigenous to India (De-Wet *et al*., 1983) [4] grown for grain and fodder purpose. It is a tetraploid (2n=4x=40) crop species. Among cultivated and wild spp., *Paspalum scrobiculatum var. scrobiculatum* is widely cultivated in India and other parts of the world as an important food crop, while *Paspalum scrobiculatum var. commersonii* is the wild spp. indigenous to India (De-Wet *et al*., 1983) [4]. Kodo millet is grown in India, Pakistan, Philippines, Indonesia, Vietnam, Thailand and West Africa (Deshpande *et al.*, 2015) [5]. It is widely distributed in damp habitats across the tropics and subtropics of the World. In India kodo millet is grown in southern Rajasthan and Maharashtra for at least 3,000 years (De-Wet *et al*., 1983) [4]. Presently it is cultivated in Uttar Pradesh, West Bengal, Kerala and Tamil Nadu (Subramanian *et al.*, 2010) [16] and some region of Maharashtra, Andhra Pradesh, Chhattisgarh, Odisha, Madhya Pradesh and consumed traditionally as health and vitality foods in rural areas. Kodo is minor crop in most of these areas, with the exception of the Deccan plateau of India (Gujarat, Karnataka and parts of Tamil Nadu), where it is grown as a major food source (Deshpande *et al.*, 2015) [5].
In India area of small millet 589.6 (000) ha. with a production of 358.9 (000) metric tone and productivity of 654.9 kg/ha. (Indian Institute of Millet Research 2014). In Chhattisgarh area of small millets 128.28(000) ha. with a production 33.90 (000) MT and productivity 264 kg/ha. In Bastar (District) area of small millets 13.00 (000) ha. with a production 3.01(000) MT and productivity 232 kg/ha (Department of Agriculture, Chhattisgarh 2014). The area under kodo millet cultivation is witnessing a declining trend in the post-green revolution period due to predominance of the major cereals such as rice and wheat. However, an intensified drive to increase the acreage of small millets is important because millets still contribute to the regional food security of the dry and marginal lands, where major cereal crops fail to yield. Nowadays, thrust to grow millets is given due to their nutritional superiority as compared to the major cereals (Sreeja *et al*., 2014) [15].
Kodo millet is gaining importance due to dual reasons like nutritional properties and stress tolerance (Kumar *et al.*, 2016) [7]. It provides low priced protein, minerals and vitamins in form of sustainable food (Yadava and Jain, 2006) [22]. Growing health consciousness among the consumers also creates demand for this type of nutri-cereals which are anti-diabetic and anti-oxidant in nature (Chandrasekara and Shahidi, 2011) [3]. Easy cultivation, negligible diseases pest, wide adaptation and drought tolerance have made these crops suitable for rainfed agriculture. It is a very hardy crop tolerant to drought and can survive on marginal soils where, other crops may not survive (Heuze *et al.*, 2012) [6].
Genetic improvement through conventional breeding approaches depends mainly on the availability of the diverse germplasm and the amount of genetic variability present in the

population (Arun Prabhu *et al.*, 2008) [2]. The knowledge of characters influencing divergence is an important aspect for a breeder. Information on the nature and degree of genetic divergence would help the plant breeder to choose right parents for breeding programmes (Vivekanandan and Subramanian, 1990) [20]. Among the multivariate procedures, a method suggested by Mahalanobis (1936) [9] known as Mahalonobis D2 statistics important tool in plant breeding and genetics for the study of genetic divergence. D2 statistics measure the source of differentiation in inter-cluster and intra-cluster levels and therefore helps in genetically divergent parent's selection in hybridization programme.

Materials and methods

The present study was carried out at Research cum Instructional Farm of S.G. College of Agriculture and Research Station Kumhrawand, Jagdalpur, Chhattisgarh. Jagdalpur is situated in 19°4'0" N and 82°2'0" E. The city is nestled on the Bastar Plateau and is positioned at a height of around 552 meters from the mean sea level. The investigation was conducted during *kharif* 2017-18 in randomized block design. With 80 germplasm of kodo millet in which 33 were selected for genetic analysis presented in table 1. The crop was sown on plot size 2.25m x 3m and the spacing between row to row is 22.5 and plant to plant is 7.5 cm. The regional crop production practices was followed. Observations were recorded on randomly chosen five plants from each genotype and both replication for 7 quantitative traits *viz.* plant height, number of productive tillers per plant, number of panicles per plant, panicle length, grain yield, fodder yield and test weight from both replication, except flowering and maturity, they were recorded on plot basis. Genetic divergence was estimated by multivariate analysis using Mahalanobis D2 (1936) [9]. Genotypes were grouped into different clusters according to Tochers method given by Rao (1952) [12].

Result and discussion

Genetic divergence analysis

The concept of D2 statistics was originally developed by P. C. Mahalanobis in 1936. He used this technique in the study of Anthropometry and Psychometry.

Quantification of genetic diversity within and between a groups of germplasm is useful in proper choice of parents for realizing higher heterosis and obtaining useful recombination. D2 statistics is important tool in plant breeding and genetics for the study of genetic divergence. It plays important role in plant breeding because hybrid between lines of diverse origin, generally display a greater heterosis than those between closely related parents. Rao (1952) [12] suggested the application of this technique for the assessment of genetic diversity in plant breeding.

1. Cluster formation

33 genotypes of kodo millet under study were grouped into 6 clusters. The clusters along with genotypes included in them are presented in table 2 The cluster V was largest with 11 genotypes followed by cluster IV (7genotypes), cluster II (6 genotypes) cluster III and cluster I were contain 4 genotypes, followed by cluster VI (1genotypes) had the lowest number of genotypes. Earlier Sao *et al.* (2016) [13] grouped twenty seven kodo millet entries into 4 different clusters through Euclidian clustering and in similar way Nirubana *et al.*, (2017) [11] grouped 103 kodo millet germplasm accessions into 11 different clusters. Clustering is a means of relative genetic closeness of genotypes. The genotypes included in same cluster are considered to be more closely related by their origin and ancestry, compare to genotype of other cluster. Clustering is important in crop improvement, for getting diverse parents in combination and transgressive breeding, and it is advised that parents should be opted from different clusters. The basic idea behind formation of clusters is to get the intra and inter cluster distances. This use as index for parents with diverse origin. The intra and inter cluster values are means derived from D2 values of cluster elements. The crossing between the genotypes placed in clusters with large inter cluster distance will be more correct approach to get desirable result (Suryanarayana *et al.*, 2014 and Kumari and Singh 2015) [17, 8].

2. Intra and inter-cluster distances

Formation of cluster and estimation of inter and intra-cluster divergence provides a basis for selection of genetically diverse parents belonging to different clusters. It is assumed that the statistical distance (D) is the index of genetic diversity. The minimum intra cluster distance was found in cluster V (0.00) and cluster VI (0.00) followed by cluster III (34.72) and cluster I (41.15). The cluster V and cluster VI exhibited zero intra cluster distance. Due to solitary nature presented in table 3. The maximum intra cluster distance was found in cluster IV followed by cluster II indicate that the genotype present, have considerable genetic distance among them. Alternatively lower intra-cluster distance indicates relative genetic closeness of genotypes (Suryanarayana *et al.*, 2014) [17]. The maximum inter cluster distance was found between cluster I and cluster VI (1585.21), followed by cluster IV and cluster VI (1349.59) showing high degree of genetic diversity indicating that genetic makeup of genotypes falling in this cluster may be entirely different from one another and thus may be utilized for future breeding programme for getting high yielding genotypes, therefore the genotypes of most diverse cluster may be used as parents in hybridization programmes to develop high yielding varieties (Nirubana *et al.*, 2017) [11]. The minimum inter cluster distance was found between cluster II and cluster III (164.63) followed by cluster II and cluster V (165.15) indicating genotypes of these clusters are comparatively genetically diverse. Thus crossing in these genotype may not produce higher amount of heterotic expression in F1's and wide range of variability in subsequent segregating (F2) populations. The next minimum inter cluster distance was found between cluster I and cluster V (173.70), cluster II and cluster IV (211.45). The clustering pattern has clearly indicated that lack diversity present between these genotypes. Wolie and Batele (2013) [21] reported highest average inter cluster divergence was (D2 = 1280), between cluster VII and cluster VIII, showing wider genetic diversity among these clusters produces individuals exhibited greater heterosis. Inter-cluster distance was higher than the intra-cluster showing wider genetic divergence between among genotypes off different clusters, to the character considered. Hence combination with wide heterotic response and superior recombination may be found by hybridization between genotypes across the clusters. Minimum intra-cluster distance were indicates narrow genetic variation with in a cluster. The clustering of genotypes clearly suggested that the higher diversity between included genotypes. Earlier Nirubana *et al.*, (2017) [11] studied intra and inter cluster divergence in kodo millet and found considerable variability between cluster III and X.

3. Cluster mean value for different characters

The cluster mean performance for each of nine characters of among test genotypes are presented in table 4 Considerable differences between clusters were observed for most of the characters studied which included plant height, tillers per plant, panicles per plant, panicle length, days to 50% flowering, days to maturity, grain yield per plot, fodder yield per plot and test weight. Cluster I included 4 genotypes (BK-35, BK-43, BK-46, BK-12), possessing highest mean value for panicles per plant (3.000), days to maturity (121.500 DAS) and lowest mean value for grain yield per plot (1.387 kg), test weight (7.820 g). Earlier Sao *et al.*, (2016) [13] reported that Cluster I had exhibited higher mean values for days to maturity, days to flowering and plant height. Cluster II contain 6 genotypes (BK-21, BK-34, BK-45, BK-48, PCGK-12, BK-2) with highest average value for tillers per plant (5.333), panicle length (7.383 cm), days to maturity (121.500 DAS), test weight (10.167g) and had lowest mean value for plant height (37.333 cm) and fodder yield per plot (10.400 kg). Cluster III contain 4 genotype (BK-64, BK-81, PCGK-12, BK-14) it not possessed first position for any character while lowest mean for panicle length (5.791 cm). Cluster IV contain 7 number of genotypes (BK-36, BK-38, BK-49, BK-1, BK-3, BK-10, BK-11) which possessed first position for panicles per plant (3.000), days to maturity (121.500 DAS), panicles per plant (3.000) and lowest mean value for tillers per plant (3.000). The cluster V retained a total of 11 genotypes including both checks *i.e.* (BK-19, BK-20, BK-42, BK-50, BK-5, BK-6, BK-7, BK-8, BK-9, IK-01, IK-02*). The cluster V had highest mean value for plant height (49.250 cm), and lowest mean value for days to maturity (97.818 DAS). The cluster VI contains 1 genotype (BK-13) possessed first position for grain yield per plot (1.603 kg) and fodder yield per plot (1.603 kg), while lowest mean value for tillers per plant (3.000) and panicles per plant (2.750).

In the present study, genotypes from cluster II selected for dwarf genotypes, cluster IV for earliness in flowering, cluster V for early maturity, cluster I and cluster IV for genotype with maximum panicles per plant, cluster II more number of tillers per plant and genotypes with maximum panicle length, cluster VI for higher grain yield per plot and for maximum fodder yield per plot and cluster I for genotype with greater test weight. The result indicates that genotypes having high values for particular character could be selected and used in the breeding programme for improvement of that character. From these clusters having high average value for grain yield per plot may be directly selected and used as parent in future breeding programme. Earlier Sao *et al*., 2016 [13] studied inter and intra cluster divergence in kodo millet and found variability for most of the trait.

4. Contribution of characters towards genetic divergence (per cent)

The contribution of the characters towards the genetic divergence is presented in table 5 and figure: 1. Out of the nine characters evaluated, days to maturity contributed maximum towards diversity (66.29%), followed by days to 50% flowering (19.70%), fodder yield per plot (5.11%), tillers per plant (4.36%), test weight (1.52%), grain yield per plot (1.14%). The characters panicle length (0.95%), plant height (0.57%) and panicles per plant (0.38%) showed very less contribution to diversity. Earlier Nirubana *et al.* (2017) [11] reported maximum contribution in the manifestation of genetic divergence for days to 50 per cent flowering, grain yield per plant, flag leaf width, number of basal tillers and plant height suggesting scope for improvement in these characters. Kumari and Singh (2015) [8] reported that maximum contribution in the manifestation of genetic divergence was exhibited by days to fifty per cent flowering followed by days to maturity, gain yield per plant, panicle length, harvest index, grain weight of main panicle, fingers per panicle, flag leaf area, number of tillers per plant and 1000-grain weight suggesting scope for improvement in these characters. Similar results have been reported earlier for days to flowering (Ulaganathan *et al.*, 2013) [18], for grain yield (Selvi *et al*., 2014) [14] and for ear width, number of tillers and finger number (Mahanthesha *et al*. 2017) [10].

The character days to maturity followed by days to 50% flowering, fodder yield per plot, tillers per plant, test weight, grain yield per plot contributed greater towards divergence. Hence, the performance of the genotypes and the traits with maximum contribution towards divergence should also be look at for improvement of kodo millet. These results are in conformity with Nirubana *et al.* (2017) [11] in kodo millet and Kumari and Singh (2015) [8] in finger millet. The parent's selection for future breeding programme will be based on the magnitude of genetic distance, cluster means magnitude for different clusters and participation of different traits towards total divergence. Genotypes that located in distinct clusters are advised to be used in breeding programme to obtain wide range of variability among segregating generation.

Table 1: List of selected 33 genotypes of kodo millet for genetic analysis

S.N.	Genotype name	S.N.	Genotype name	S.N.	Genotype name
1.	BK-19	12.	BK-48	23.	BK-6
2.	BK-20	13.	BK-49	24.	BK-7
3.	BK-21	14.	BK-50	25.	BK-8
4.	BK-34	15.	BK-64	26.	BK-9
5.	BK-35	16.	BK-81	27.	BK-10
6.	BK-36	17.	PCGK-8	28.	BK-11
7.	BK-38	18.	PCGK-12	29.	BK-12
8.	BK-42	19.	BK-1	30.	BK-13
9.	BK-43	20.	BK-2	31.	BK-14
10.	BK-45	21.	BK-3	32.	IK-01*
11.	BK-46	22.	BK-5	33.	IK-02*

Table 2: Cluster formation of kodo millet genotypes

Cluster	Number of genotypes	Genotypes
I	4	BK-35, BK-43, BK-46, BK-12,
II	6	BK-21, BK-34, BK-45, BK-48, PCGK-12, BK-2
III	4	BK-64, BK-81, PCGK-8, BK-14
IV	7	BK-36, BK-38, BK-49, BK-1, BK-3, BK-10, BK-11
V	11	BK-19, BK-20, BK-42, BK-50, BK-5, BK-6, BK-7, BK-8, BK-9, IK-01*, IK-02*
VI	1	BK-13

Table 3: Inter and intra cluster distance

		Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V	Cluster VI
Cluster	I	41.148	247.645	576.110	234.515	173.700	1585.213
Cluster	II		78.864	164.625	211.445	165.154	779.960
Cluster	III			34.732	450.784	265.154	359.643
Cluster	IV				206.177	384.551	1349.589
Cluster	V					0.000	881.641
Cluster	VI						0.000

Table 4: Cluster mean performance for different characters of kodo millet

Cluster	Plant Height (cm)	Tillers/Plant	Panicles/Plant	Panicle Length (cm)	Days to 50% Maturity (das)	Days to Maturity (das)	Grain Yield kg/Plot	Fodder Yield kg/Plot	1000 Grain Weight (g)
I	49.727	3.727	2.909	6.391	66.818	97.818	1.391	11.445	8.164
II	42.786	3.571	2.821	6.100	71.929	111.500	1.435	11.729	7.943
III	37.333	5.333	2.833	7.383	75.000	121.500	1.383	10.400	10.167
IV	45.000	3.000	3.167	7.017	80.000	113.500	1.368	14.433	7.367
V	52.500	4.500	3.000	6.500	60.500	99.000	1.180	11.000	10.050
VI	47.500	3.000	3.000	8.400	65.500	121.500	1.400	14.800	9.350

Table 5: Contribution of each character of individual characters

	Characters	Contribution %
1.	Plant Height (cm)	0.57
2.	Tillers/Plant	4.36
3.	Panicles/Plant	0.38
4.	Panicle Length (cm)	0.95
5.	Days to 50% Maturity (das)	19.70
6.	Days to Maturity (das)	66.29
7.	Grain Yield kg/Plot	1.14
8.	Fodder Yield kg/Plot	5.11
9.	1000 Grain Weight (g)	1.52

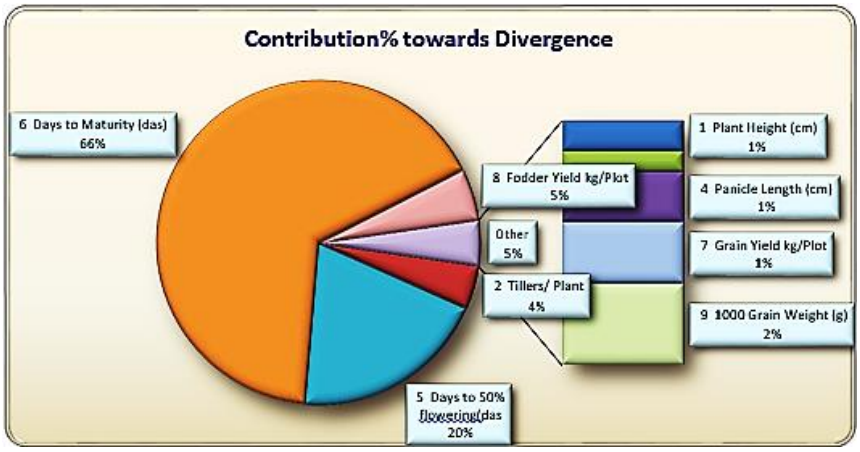

Fig 1: Contribution of each of the characters to the divergence

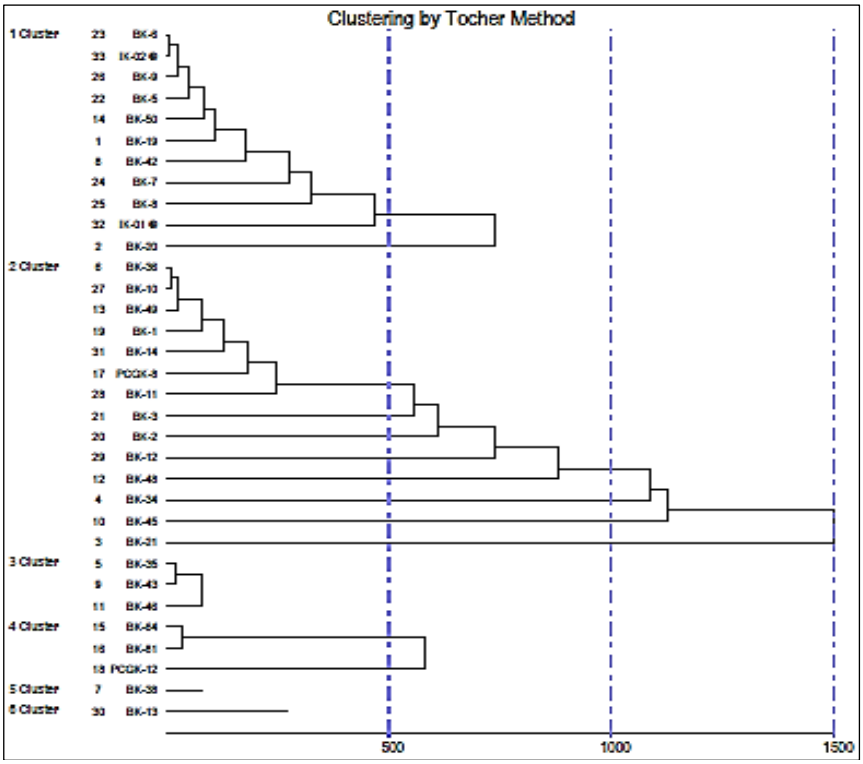

Fig 2: Dendrogram showing clustering pattern of 33 genotypes

Conclusion

Considerable amount of genetic divergence was present among 33 genotypes. These genotypes were groups into six clusters the maximum cluster distance observed for cluster I including four genotypes BK-35, BK-43, BK-46, BK-12 and cluster VI including 1 genotype BK-13. These two clusters exhibited higher genetic diversity and thus genotypes of these cluster may be used for inter varietal hybridization programme for getting higher yielding recombinants. The character days to maturity contributed maximum towards diversity followed by days to 50% flowering, fodder yield, tillers per plants and other characters contributed 5% towards diversity.

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