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Original Research Article

Assessment of Genetic Diversity of Indigenous Germplasm Accession of Kalmegh [*Andrographis paniculata* (Burm. F.) Nees]

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A B S T R A C T

Kalmegh [*Andrographis paniculata* (Burm. F.) Nees] is a medicinal plants of immense the rapeutic value. The present study was aimed to elucidate its genetic diversity based on morph-chemical. The study material consists of 134 accessions belonging to 12 ecogeographic regions. These 134 accessions were grown for characterization, evaluation and identification of trait-specific germplasm using 16 important quantitative traits viz., days to 50% flowering, plant height (cm), stem diameter (cm), no. of secondary branches plant^{-1}, no. of tertiary branches plant^{-1}, leaf length (cm), leaf width (cm), petiole length (cm), no. of nodes plant^{-1}, internodes length (cm), seed pod^{-1}, pod length (cm), chlorophyll content (SPAD), herbage fresh weight plant^{-1} (g), herbage dry weight plant^{-1} (g) and andrographiliode. D^2 statistics revealed significant differences in all the metric traits and sufficient inter and intra cluster distances indicating considerable diversity among the accessions. Maximum intra-cluster distance was noted in cluster XI (531.957) and highest inter cluster distance was observed in cluster-VII and X (1882.208). D^2 cluster analysis also revealed that various accessions available in different eco-geographic regions might have originated from native places. The inter cluster distance in present studied higher than intra cluster distance in most of the cases reflecting wider diversity among the genotypes of distant group. Hence, inter crossing of genotype from diverse cluster showing high mean performance will be helpful in obtaining better recombination with higher genetic variability.

Keywords

Andrographis paniculata, medicinal plant, Genetic diversity, D^2 statistics

Introduction

Kalmegh [*Andrographis paniculata* (Burm. F.) Nees], family Acanthaceae, is a medicinal herb, commonly known as 'King of Bitter' Maha-tita or Bhui-neem. *A. paniculata* is much smaller in size, but has a bitter taste similar to neem (*Azadirachta indica* A. Juss.) (Niranjan *et al.,* 2010). It is placed at 17th position among the 32 prioritized medicinal plants of India. Traditionally, this herb is used as anti-inflammatory, anti-bacterial, anti-oxidant, anti-parasitic, anti-spasmodic, anti-diabetic, anti- carcinogenic, anti-pyretic, anti-diarrheal, nematocidal, anti HIV and hepatoprotective drugs with wide geographic distribution from the peninsular of India, Sri Lanka, South-east Asia, China, America and West Indies to the Christmas Island in Indian ocean (Kumar *et al.,* 2012; Lattoo *et al.,* 2008). South India and Sri Lanka possibly represent the origin and diversity centers of *A. paniculata* because native populations of plants are found throughout these areas. *A. paniculata* (Burm. F.) Nees, is a diploid (2n = 2x = 50) species usually found in wild but under cultivation

in India. Plant is an erect herb, grows up to the height of 30–110 cm under favorable conditions. The stem is quadrangular with more branches. The flower is zygomorphic, complete, pentamerous, hypogynous, pedicellate, and bisexual. Stigma is closely attached to the anthers till the flower opening and promotes the mechanism of self-pollination (Sharma and Jain, 2015). The corolla is white with maroon streak on upper lip which attracts the insect pollinators like honey bees, butter flies etc. The fruit is capsule, linear, oblong and acute at both the ends. The seeds are small, sub quadrate and yellowish brown in colour and slightly smaller than mustard seed. The seeds per capsule are numerous and orthodox in nature. The plant is indeterminate and exhibits non synchronous maturity and seeds dispersed regularly through capsule dehiscence. The seeds of kalmegh are dormant due to hard seed coat. Evaluation and cataloguing of genetic variability is necessary for optimum genetic enrichment and effective conservation of the allelic and genotypic variability. Genetic diversity analysis based on morphological and biochemical traits is extremely useful. The limited knowledge about genetic diversity in *A. paniculata* provides the rationale for this study, which provides methods for determining intraspecific relatedness for the selection of different genotypes for breeding, effective conservation and management of its germplasm resources. Now a day, it is also used in the form of "Andrographis gel" for treating periodontitis. Furthermore, the plants are also used as feed supplement in large scale industrial chicken and pig farms. (Maison *et al.,* 2005). In crop improvement programme, genetic variability for agronomic traits as well as quality tests in almost all the crops is important, since this component is transmitted to the next generation (Singh, 1996).

Materials and Methods

The present study was conducted during *Kharif* 2014-15 and 2015-16, the mean annual rainfall during crop growing season measures 1057.4 and 998.3mm respectively. The material for present study comprised 134 accessions a long with 2 check Anand Kalmegh-1 (Directorate of Medicinal and Aromatic Plant Board) and Simmegha (Central Institute of medicinal and Aromatic plant) procured from different eco geographic regions of Chhattisgarh and maintained in the germplasm repository at Raipur.

Data were recorded on 134 competitive plants for each accession for series of 16 metric traits: days to 50% flowering, plant height (cm), stem diameter (cm), no. of secondary branches plant^{-1}, no. of tertiary branches plant^{-1}, leaf length (cm), leaf width (cm), petiole length (cm), nodes plant^{-1}, internodes length (cm), no. of seeds/pod^{-1}, pod length (cm), chlorophyll content (SPAD), herbage fresh weight plant^{-1} (g), herbage dry weight plant^{-1} (gm) and andrographiliode. The data were subjected to analysis of variance and only those characters in which variation observed was significant were considered for multivariate analysis of D^2 statistics based on Mahalanobis (1936) and Rao (1952). The analysis was done by cluster formation was confirmed by Tocher's method. The relative contribution of each character towards genetic divergence was also worked out.

Results and Discussion

The choice of genetically diverse parents for hybridization is an important feature of any crop improvement programme for getting desirable segregates. The multivariate analysis based on Mahalanobis D^2 or non-hierarchical Euclidean cluster analysis is

used for divergence analysis. The D^2 analysis classifies the genotypes into relatively homogeneous groups in such a way that within cluster diversity is minimized and between clusters diversity is maximized. The respective genotypes from diverse cluster can be utilized in breeding programme depending upon breeding objectives. The results of earlier studied are relevant only for the material and environmental involved in a particular study and cannot be generalized. Therefore, study on genetic divergence on the available germplasm under the environment where it is to be exploited is essential for successful utilization of available resources.

Genetic diversity is an important factor and also a prerequisite in any hybridization programme. Multivariate analysis by mean of Mahalanobis D^2 statistic is a powerful tool in quantifying the degree of divergence at genotypic level. Vavilov (1926) was the first to emphasize the need for a really broad genetic base for crop improvement. A set of 134 accession along with 2 checks of kalmegh were subjected to D^2 analysis for 16 characters based on D^2 values twelve cluster were formed (Table4.3). This indicated that substantial diversity existed in the all the genotypes evaluated in the present study.

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This is an agreement with earlier reports indicating substantial diversity in kalmegh materials. This present study also suggests that, there is no relationship between geographical and genetic diversity as genotype chosen from different eco-geographical regions are grouped in different clusters. The cluster III were the largest which consisted of 47 genotype, followed by cluster IV, I, XI, II (32, 23, 14, 11 genotypes respectively), cluster V, VI, VII, VIII, IX, X and XII (1 genotypes). From the clustering pattern, it was found that the genotypes from different region were independent of their genetic origin. Hence the genotype Studied are reliable enough for hybridization and selection.

Maximum intra-cluster distance was noted in cluster XI (531.957) followed by cluster IV (311.702), cluster III (274.762), cluster II (155.228), cluster I (73.392), while minimum intra-cluster distance was recorded in cluster V, cluster VI, cluster VII cluster VIII, cluster IX, cluster X, and cluster IX (0.000). The inter and intra cluster distance among the twelve cluster are presented in table 4.4 and fig 4.1. Maximum inter-cluster distance was calculated between cluster VII and X (1882.208) followed by cluster VII and XII (1854.556), cluster VI and XII (1745.212), cluster XI and XII (1588.499), cluster VIII and X (1555.442), cluster VIII and XII (1552.625), cluster VII and XI (1425.871), cluster IV and XII (1407.880), cluster II and VII (1247.510), cluster IV and X (1201.658), cluster V and VII (1180.310), cluster IX and X (1123.204), cluster II and IV (1087.083), cluster IV and XI (1063.154), cluster X and XII (1054.775), cluster II and XII (1043.669), cluster IX and XI (1040.515), cluster VIII and XI (1023.083), cluster IV and V (1016.159). This suggested that the hybridization programme involving parents from these cluster is expected to give higher

frequency of better segregates or desirable combination for development of useful genetic stocks or varieties. However, minimum distance was found between inter-cluster v and XII (254.614) followed by inter-cluster VII and VIII (267.055) and cluster I and III (284.589) indicating minimum diversity (differences) for the genes under study.

Table.1 Average intra-cluster and inter cluster distance (D^2 values) among the 12 cluster in Kalmegh

	1 Cluster	**2 Cluster**	**3 Cluster**	**4 Cluster**	**5 Cluster**	**6 Cluster**	**7 Cluster**	**8 Cluster**	**9 Cluster**	**10 Cluster**	**11 Cluster**	**12 Cluster**
1 Cluster	(73.392)	729.786	284.589	316.894	524.222	628.926	490.152	520.766	551.527	857.021	853.795	822.243
2 Cluster		(155.228)	429.049	1087.886	427.089	425.832	1247.510	785.855	507.957	327.350	601.741	1043.669
3 Cluster			(274.762)	565.489	430.853	454.075	767.239	575.963	482.403	575.750	676.932	868.134
4 Cluster				(311.702)	1016.159	744.600	712.877	749.196	996.224	1201.658	1063.154	1407.880
5 Cluster					(0.000)	894.054	1180.310	835.100	286.361	655.044	963.970	254.614
6 Cluster						(0.000)	879.472	416.169	691.325	806.992	544.941	1745.212
7 Cluster							(0.000)	267.055	610.928	1882.208	1425.871	1854.556
8 Cluster								(0.000)	290.771	1555.442	1023.083	1552.625
9 Cluster									(0.000)	1123.204	1040.515	761.852
10Cluster										(0.000)	707.962	1054.775
11Cluster											(531.957)	1588.499
12Cluster												(0.000)

Table.2 Mean performance of genotype in individual cluster for different yield traits

	Days to 50% Flowering	**Plant Height(cm)**	**Stem Diameter (cm)**	**No. of secondary Branches/ Plant**	**No of tertiary Branches/ Plant**	**Leaf Width (cm)**	**Leaf Length (cm)**	**Petiole Length (cm)**	**No. of nodes/ Plant**	**Internodes Length (cm)**	**No. of seeds/Pod**	**Pod Length (cm)**	**Chlorophyll Content (SPAD)**	**Herbage Fresh Weight (g)**	**Herbage Dry Weight (g)**	**Andographiloide (mg/G)**
1Cluster	120.457	65.738	2.088	32.239	107.761	1.549	6.140	1.089	20.717	2.572	11.163	1.779	56.255	31.135	23.395	1.157
2Cluster	139.091	94.986	2.632	35.409	205.795	1.478	6.191	0.980	23.841	2.891	11.577	1.848	57.799	46.966	40.545	0.517
3 Cluster	127.410	78.927	2.439	34.983	149.737	1.597	6.786	1.008	22.438	2.771	11.296	1.809	57.167	38.874	32.618	0.932
4 Cluster	121.328	63.986	2.099	31.977	121.156	1.455	6.307	0.949	21.523	2.630	11.354	1.830	55.194	28.821	21.353	1.430
5 Cluster	121.000	102.600	2.250	39.000	123.250	1.605	8.500	1.160	24.750	2.750	11.835	1.785	58.205	41.530	37.335	0.440
6 Cluster	149.000	96.950	2.525	31.750	211.000	2.005	7.930	1.310	23.250	2.850	10.920	1.890	56.900	44.610	38.245	1.225
7 Cluster	156.000	38.025	1.575	22.500	80.500	1.060	6.250	0.730	16.500	1.750	10.500	1.675	63.260	15.180	10.340	1.140
8 Cluster	163.000	86.675	1.675	33.250	121.000	1.415	5.465	1.050	21.750	3.025	11.665	1.865	53.275	38.570	31.260	1.080
9 Cluster	150.500	92.800	2.350	34.750	106.250	1.580	7.550	1.275	24.500	2.900	11.085	1.875	57.300	50.010	41.835	0.530
10 Cluster	112.000	81.850	2.925	30.250	225.000	0.780	5.950	0.920	25.000	2.725	11.165	1.815	57.885	45.675	39.160	0.565
11 Cluster	133.214	92.216	2.645	35.696	210.625	1.504	5.968	1.101	23.321	3.109	11.185	1.879	55.028	43.700	36.449	0.969
12 Cluster	98.000	109.400	2.375	42.250	83.500	1.545	5.550	2.840	24.000	2.850	11.085	1.695	63.965	50.215	43.230	0.435

Fig.1 A dendrogram based Tocher method on clustering of 134 accessions of *A. paniculata* using the genetic distance estimates

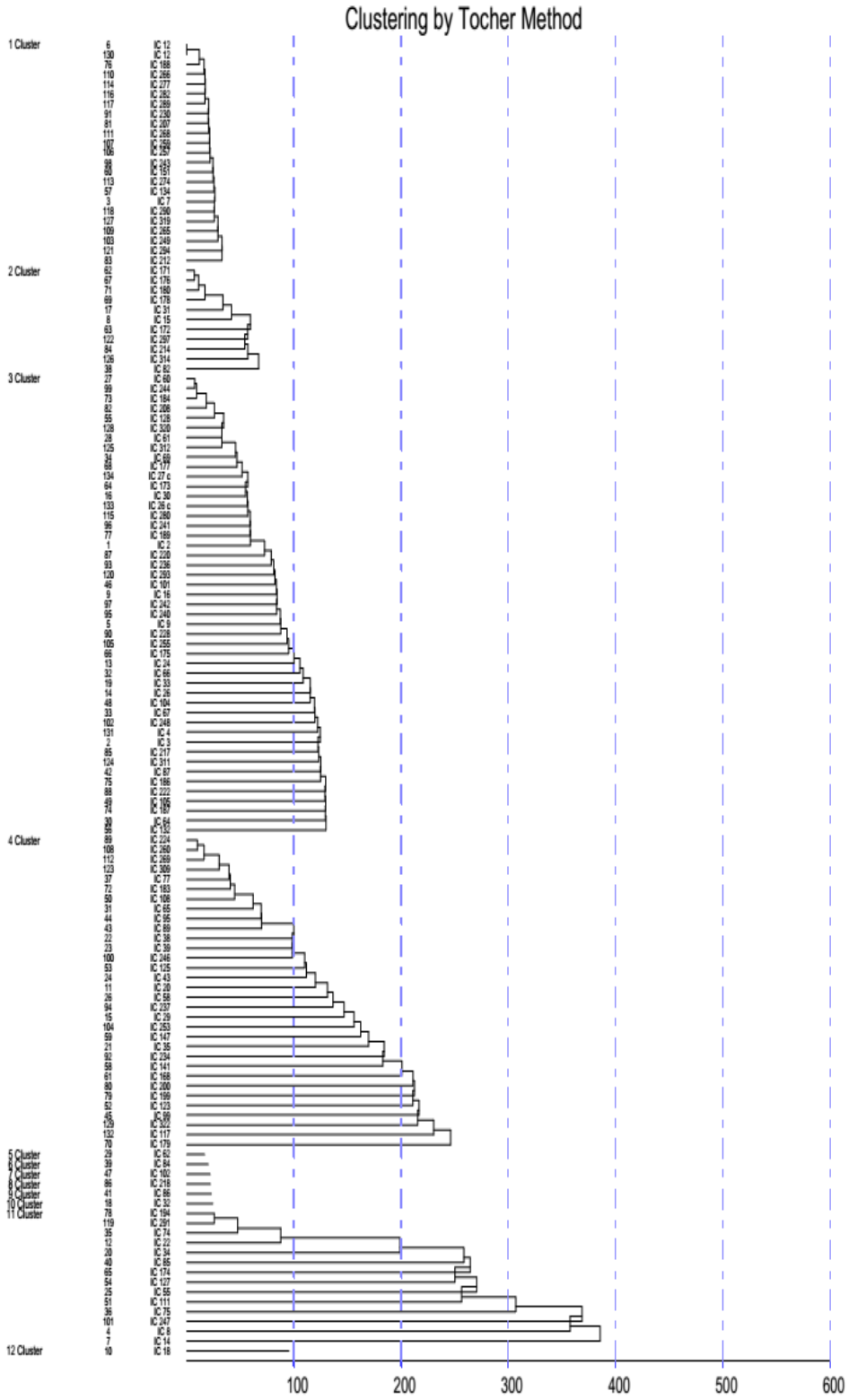
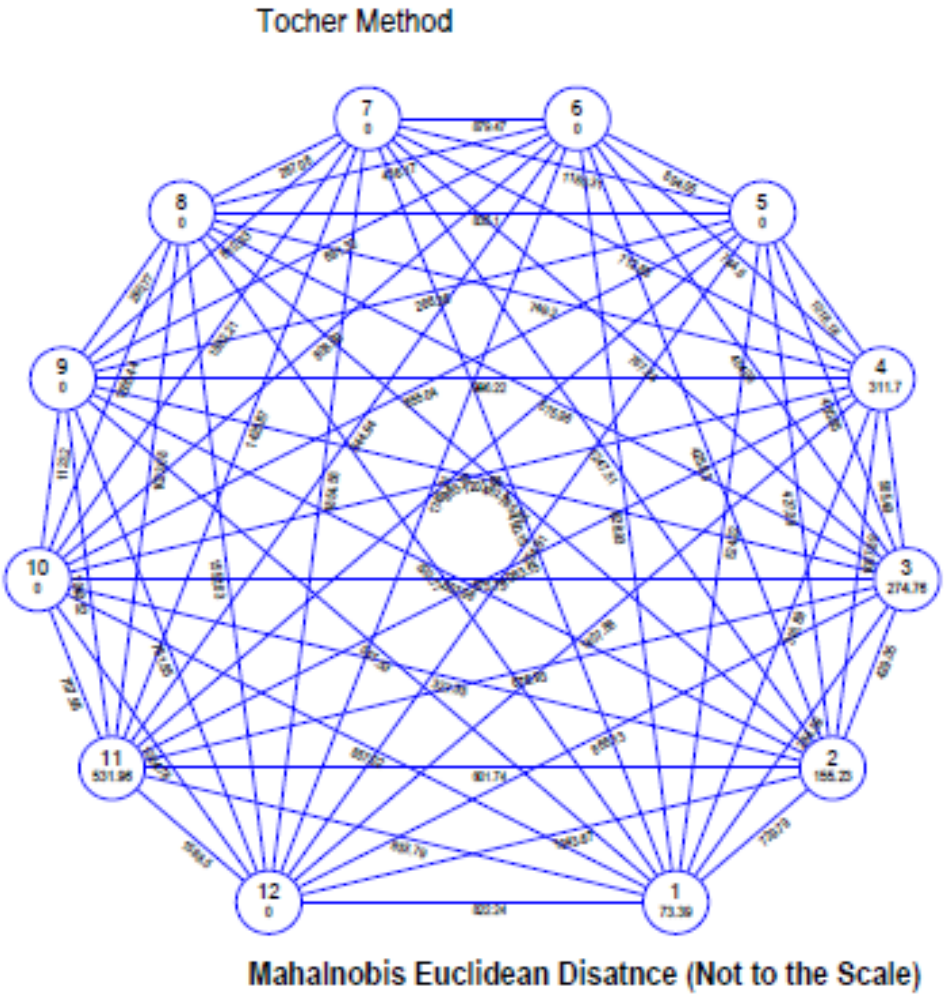


Fig.2 Mahalanobis generalized distance through Tocher Method



Cluster mean

The mean values for different character were compared across the cluster and are presented in Table 4.5. Cluster XII having maximum intra cluster distance (divergence) had the highest dry herbage weight (43.230 g) followed by Cluster IX (41.835), Cluster II (40.545), Cluster X (39.160), Cluster VI (38.245).

Overall observation of cluster means indicate that cluster XII having maximum traits and high cluster mean value plant height (109.400), no. of secondary branches plant^{-1} (42.250), petiole length (2.840), chlorophyll content (63.965), herbage fresh weight (50.215). Cluster X shown higher cluster mean values for stem diameter (2.925), no. of tertiary branches plant^{-1} (225.000), no. of nodes plant^{-1} (25.00). Cluster VI shown higher cluster mean values for leaf width (2.005), pod length (1.890).

Cluster V shown higher cluster mean values for leaf length (8.500) and no. of seeds pod^{-1} (11.835). Cluster VIII shown higher cluster mean values for days to 50% flowering (163.00). Cluster XI shown higher cluster mean values for internodes length (3.109). Cluster IV shown higher cluster mean values for andrographiloide (1.430).

Results indicated that, the genotypes from most distant clusters may be utilized as parents in crossing programme to isolate desirable segregate for herbage dry weight. In present study 134 accessions shown considerable divergence among the genotypes for different characters. Based on the result obtained from the present study, it is concluded that the mean values of cluster for different character and per se performance of the genotypes grouped in respective cluster could be selected (Table-2) for a viable hybridization programme for improving a particular character.

The pattern of distribution of kalmegh genotypes in various clusters revealed existence of considerable diversity present in the material (Table-2). The highest intra cluster distance was observed for cluster XI. Hence, genotypes belonging to this cluster *viz.*,IKM-7, IKM-9, IKM-104, IKM-151, IKM-207, IKM-224, IKM-230, IKM-243, IKM-259, IKM-260, IKM-265, IKM-268, IKM-280, IKM-290, IKM-294, IKM-312, IKM-319, IKM-320, IKM-26C may be utilized as parent in future breeding programme with the genotype belonging to cluster IV *i.e.,* IKM-18, IKM-33, IKM-77, IKM-89, IKM-95, IKM-101, IKM-108, IKM-147 and IKM-83 as the maximum inter cluster distance was noted between the cluster VII and X. This suggested that the hybridization programme involving parents from these cluster is expected to give higher frequency of better segregates or desirable combination for development useful genetic stocks pipelines or varieties for Chhattisgarh plains.

Cluster analysis revealed that the 134 genotype of kalmegh were grouped in twelve clusters. Considerable amount of genetic divergence was present among134 genotype. The cluster III were the largest which consisted of 47 genotype, followed by cluster IV, I, XI, II (32, 23, 14, 11 genotypes respectively), cluster V, VI, VII, VIII, IX, X and XII (1 genotypes). Maximum intra-cluster distance was observed in cluster XI followed by cluster IV, cluster III, cluster II, cluster I, while minimum intra-cluster distance was recorded in cluster V, cluster VI, cluster VII cluster VIII, cluster IX, cluster X, and cluster IX and the highest inter cluster distance was observed in cluster-VII and X. This suggested that the hybridization programme involving parents from these cluster is expected to give higher frequency of better segregates or desirable combination for development of useful genetic stocks or pipeline or varieties for Chhattisgarh. The inter cluster distance in present studied higher than intra cluster distance in most of the cases reflecting wider diversity among the genotypes of distant group. Hence, inter crossing of genotype from diverse cluster showing high mean performance will be helpful in obtaining better recombination with higher genetic variability.

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