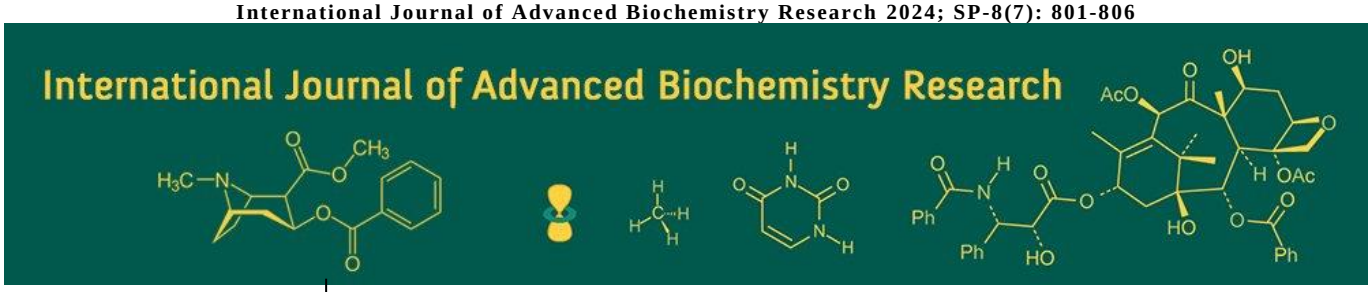

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Genetic diversity analysis using Mahalanobis's D^2 and Principal component analysis of aloe vera (*Aloe barbadensis* L.)

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Abstract
In present investigation carried out study the genetic diversity in thirty-seven *Aloe barbadensis* accessions along with check (GAKP-1) were evaluated at research cum instructional farm of Dept. of Genetics & Plant Breeding, IGKV, Raipur during 2023-24 in RCBD in two replication. The genetic diversity was analysed through Mahalanobis's D^2 statistics and principal component analysis (PCA). The Mahalanobis's D^2 statistics revealed that the maximum inter cluster distance was observed in between VI and VII (266.9) have single accession CGAV-19 and CGAV-35 respectively which depicts hybridization involving these two diverse parents expected to produce greater frequency of better segregates in desirable combination for improvement of genetic stock and development of variety. The PCA revealed that out of ten only three principal components (PCs) exhibited >1 Eigen value, and showed about 78% variability among the traits studied. The PC-I shows 42.42% followed by PC-II showed 23.68% and PC-III exhibited 11.94% and account for 78% variability for traits under study. The first PC accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible. The present study depicted that morphological trait is useful in preliminary evaluation to access genetic diversity among morphological distinct aloe vera genotype.

Keywords: *Aloe barbadensis*, cluster analysis, principal component analysis

Introduction
Aloe (*Aloe barbadensis* L.) is a xerophytic perennial plant which belong to family Liliaceae. The name aloe vera was derived from the *Arabic* word '*alloeh*' which means 'shining bitter substance' (Deshmukh *et al*., 2019) [3]. In India, it is found in Maharashtra, Andhra Pradesh, Gujarat, Rajasthan, Tamil Nadu and Uttar Pradesh (Farming India, 2019) [4]. This perennial succulent plant has the ability to develop water storage tissue in the leaves to survive in dry conditions where there is less or erratic rainfall (Kumar and Yadav, 2014) [6].
Several economically significant species of aloe, such as *A. ferox* Mill., *A. zeylanicum*, *A. Africana* Mill., *A. arborescence* Mill., and *A. perryi* Back., exist instead of Aloe Vera. (Deshmukh *et al*. 2019) [3].
The aloe vera leaf mainly consist of three layers, Rind - the outer most protective layer, Sap – the central layer consist latex, Mucilage Gel - the inner layer of the leaf that contain gel in it (Vogler and Ernst, 1999) [8].
Aloe Vera is utilized as a tonic for liver problems, anemia, and poor digestive function in India. The principal application of aloe vera is in the production of health drinks, such as tea, juices, and other beverages. Owing to its special medicinal qualities and positive impacts on people, aloe vera has become more and more common in food product formulations. Other important properties of aloe vera are anti diabetic antiseptic, antioxidant, antiulcer, hepatoprotective, immunomodulatory and wound and burn healing effect etc. Aloe has broad range of pharmacological properties, including anti-inflammatory, antiviral, antioxidative actions, antibacterial, immunostimulant, antifungal, analgesic, antitumor, antidiabetic and inhibition of tumor cells activation and proliferation (Ray *et al*., 2013) [7].
Aloin has long been used as a purgative and laxative. Acemannan is the major carbohydrate fraction in the Aloe Vera gel which shows antiviral and antineoplastic effects. Anthraquinones, carbohydrates, amino acids, organic acids, minerals and microelements,

active enzymes, and vitamins are the seven main chemical components of aloe, among which anthraquinones are the most important active ingredients and the four matters showing quite high medical values, including aloin, Aloe emodin, Aloe bitter and Aloe lectin, belong to anthraquinones (Wang, 2009) [9].

The improvement in traits can be achieved through understanding the nature and amount of variability present in the genotypes of Aloe used in breeding programs. Additionally, the evaluation will yield information regarding the distinctness and originality of genotypes, which is crucial for the best possible conservation of genotypic variability. (Bisrat *et al.*, 2000) [2].

Materials and Methods

The 37-accession collected from different district of the C.G. and state of Uttar Pradesh, Haryana & Gujrat were evaluated in Research Cum Instructional Farm, Dept. of Genetics & Plant Breeding, IGKV, Raipur during kharif 2023. The row to row spacing and plant to plant distance maintained at 30 × 30 cm in Randomized Block Design (RBD) in two replications. The present investigation was carried out to assess genetic diversity of collected accession of aloe vera.

Table 1: List of genotype accessions collected from different district of C.G

Accessions	District	Accessions	District	Accessions	District
CGAV-1	Bilaspur	CGAV-13	Raipur	CGAV-25	Dhamtari
CGAV-2	Ambikapur	CGAV -14	Durg	CGAV-26	Raipur
CGAV-3	Raipur	CGAV-15	Rajnandgaon	CGAV-27	Durg
CGAV-4	Baikunthpur	CGAV-16	Rajnandgaon	CGAV-28	Narayanpur
CGAV-5	Bhilai	CGAV-17	Manpur- mohla	CGAV-29	Ambikapur
CGAV-6	Kondagaon	CGAV-18	Rajnandgaon	CGAV-30	Kawardha
CGAV-7	Raigarh	CGAV-19	Rajnandgaon	CGAV-31	Manpur- mohla
CGAV-8	Champa	CGAV-20	Balod	NDAB-14	Ayodhya
CGAV-9	Dhamtari	CGAV-21	Bastar	NDAB-17	Ayodhya
CGAV-10	Jagadalpur	CGAV-22	Rajnandgaon	HAV05-8	Hissar
CGAV-11	Jashpur	CGAV-23	Raipur	AB-9	Gujrat
CGAV-12	Raipur	CGAV-24	Raipur	AB-18	Gujrat
				GAKP-1(Check)	

The leaves from randomly selected five plants were of each accession of aloe vera harvested and observations was recorded for quantitative and qualitative traits such as plant height (cm), leaf length (cm), leaf width (cm), leaf thickness(cm) , fresh leaf weight (g), gel weight (g), aloe gel content (%), no. of spine/leaf, aloe gel colour, plant growth habit, upper leaf surface colour, lower leaf surface colour, spine colour, leaf shape, chlorophyll a & b content (mg/g) etc. The statistical analysis of quantitative and qualitative traits was done using Randomized Block Design (RBD).

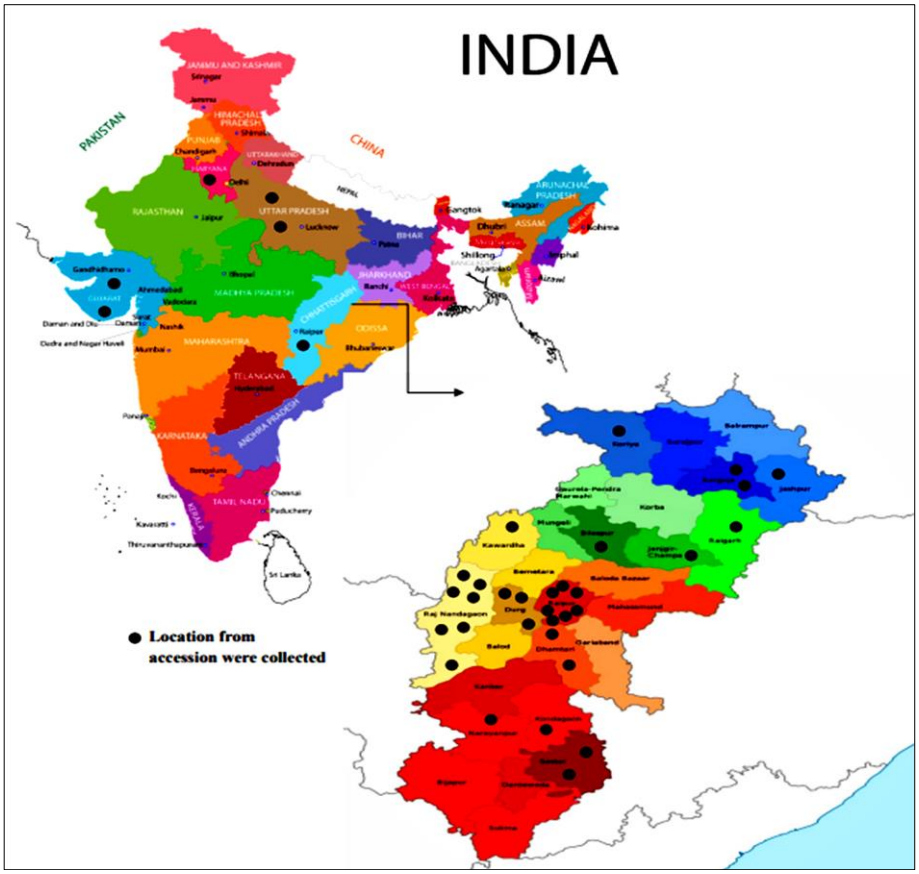

Fig 1: Map showing Aloe vera accessions collection sites from different places of India & Chhattisgarh

Results and Discussion

Cluster Analysis

In present study quantitative assessment of genetic diversity has been studied using Mahalanobis's D^2 statistics on 37 genotypes. Which are grouped into 7 cluster and presented in table 2. The maximum number of genotypes were found in cluster I consist of 23 accessions, followed by cluster II and III with 4 accession and cluster, IV and V each possess 2 accessions followed by cluster VI and VII, each represented by single accession.

Table 2: Clustering pattern of 37 genotype accessions of aloe vera

Cluster number	No. of genotype	Name of genotype
Cluster I	23	CGAV-1, CGAV -10, CGAV -11, CGAV -12, CGAV - 13, CGAV -14, CGAV - 15, CGAV -16, CGAV -17, CGAV - 2, CGAV -21, CGAV -28, CGAV -29, CGAV -3, CGAV -30, CGAV -31, CGAV -32, CGAV – 34, CGAV -36, CGAV -6, CGAV -7, CGAV-8, CGAV-9
Cluster II	4	CGAV-18, CGAV-24, CGAV-26, CGAV-27
Cluster III	4	CGAV-20, CGAV-22, CGAV-25, CGAV-4
Cluster IV	2	CGAV-23, CGAV-5
Cluster V	2	CGAV-33, GAKP-1
Cluster VI	1	CGAV-19
Cluster VII	1	CGAV-35

The inter and intra cluster distance among the 7 cluster are presented in Table 2 and Fig 2. The maximum inter cluster distance was observed in between VI and VII (266.9) followed by cluster III and VII (253.22) followed by cluster III and IV (208.94) followed by cluster V & III (178.30) , cluster VII & IV(174.08) followed by cluster VII & I (168.15) and cluster VIII and II (143.60). from the clustering pattern it was found that accessions from different region were dependent of their genetic region. Hence the hybridization made using these parents will produce better segregants and varieties. It was also reported by Kiran and Tirkey (2018) [5].

Table 3: Estimates of intra (diagonal and bold) and inter cluster distances among seven clusters.

Cluster Number	Cluster-I	Cluster-II	Cluster-III	Cluster-IV	Cluster-V	Cluster-VI	Cluster-VII
Cluster-I	35.08824	95.38878	107.5321	80.12393	84.63246	168.1502	106.8294
Cluster-II		43.81788	96.54233	100.3801	98.94095	67.46413	143.6032
Cluster -III			33.82789	208.9408	178.3059	92.89747	253.2248
Cluster-IV				32.46387	76.57993	174.0805	62.15469
Cluster-V					46.20111	148.5584	89.98727
Cluster-VI						0.004	266.9345
Cluster-VII							0.002

The mean values of clusters show wide range of variation for all 10 traits under this study. Cluster VII exhibit highest mean value for plant height (39.85), leaf width (5.45), no. of spine/leaf (24.5) While, cluster IV contained genotype with higher mean value for fresh leaf weight (132.92), gel weight (84.8) and leaf length (38.02). Cluster V contain highest mean value for traits leaf thickness (1.5), total chlorophyll content (0.43), chlorophyll -a content (0.35). cluster III showed high mean value for the trait aloe gel content**.**

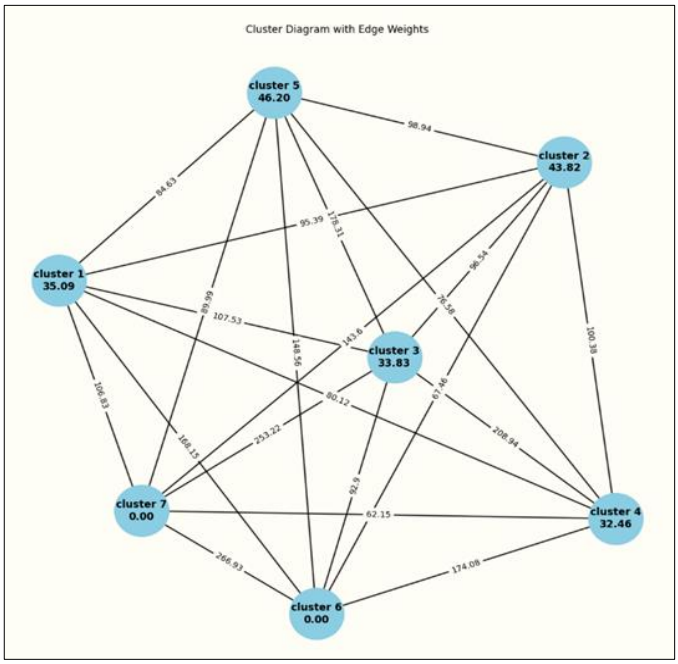

Fig 2: Cluster diagram representing inter and intra cluster distance

Table 4: Cluster mean values of seven clusters for different character

S. No.	Trait	cluster I	cluster II	cluster III	cluster IV	cluster V	cluster VI	cluster VII
1	Plant height (cm)	37.28043	39.3625	38.7	42.6	35.4	30.1	39.85
2	Leaf length (cm)	34.31739	37.75	34.425	38.025	31.6	27.6875	36.4
3	Leaf width (cm)	4.065217	4.7375	5.35	4.975	4.4	3.2875	5.45
4	Leaf thickness (cm)	0.917391	0.9875	1.125	1.175	1.5	0.8125	0.85
5	Total chlorophyll (mg/g)	0.22913	0.37625	0.215	0.2675	0.435	0.3625	0.19
6	Fresh leaf weight (g)	80.14565	110.25	110.775	132.925	113.95	54.975	66.35
7	Chlorophyll-a (mg/g)	0.155652	0.29625	0.15	0.1875	0.35	0.27875	0.135
8	No. Of spine/leaf	20.13043	20.125	19.25	24.25	21	21.25	24.5
9	Aloe gel content (%)	63.3313	66.37875	70.8425	63.735	72.4	63.6425	61.995
10	Aloe gel weight (g)	50.56522	73.0375	78.75	84.8	82.45	34.675	41.15

Principal component analysis (PCA)

In present investigation PCA was performed for gel yield and yield contributing traits of aloe vera and presented in table 4.9. Out of ten, only three principal components (PCs) exhibited >1 Eigen value, and showed about 78% variability among the traits studied. So, these three principal components were given due importance for the further explanation. The PC-I had 42.42%, PC-II showed 23.68%, PC-III exhibited 11.94% variability for traits under study. The first PC accounts for as much of the variability in the data as possible, and each succeeding component accounts for as much of the remaining variability as possible.

Table 5: Eigen value, percentage of variance, cumulative percentage of variance of the principal component

Principal component	Eigenvalue	Percentage of variance	Cumulative percentage of variance
PC-I	4.242	42.421	42.421
PC-II	2.368	23.684	66.105
PC-III	1.194	11.944	78.049
PC-IV	0.984	9.84	87.889
PC-V	0.64	6.402	94.291
PC-VI	0.261	2.611	96.902
PC-VII	0.21	2.097	98.999
PC-VIII	0.086	0.862	99.861
PC-IX	0.013	0.128	99.989
PC-X	0.001	0.011	100

The study revealed that the first three PC values contributed for yield attributing traits. The trait fresh leaf weight (0.46), aloe gel weight (0.45), leaf width (0.41), leaf length (0.386), plant height (0.389) and leaf thickness (0.33) were major contributors for 42.42% variability in PC -I.

Total chlorophyll content (0.596), chlorophyll -a content (0.592) and aloe gel content (0.355) were responsible for 23.684% variation in PC-II, whereas no.of spine/leaf (0.56), chlorophyll -a (0.33), total chlorophyll content (0.31)were accountable for 11.944% variability in PC-III.

Similar finding was recorded by Kumar *et al.* (2019) [6].

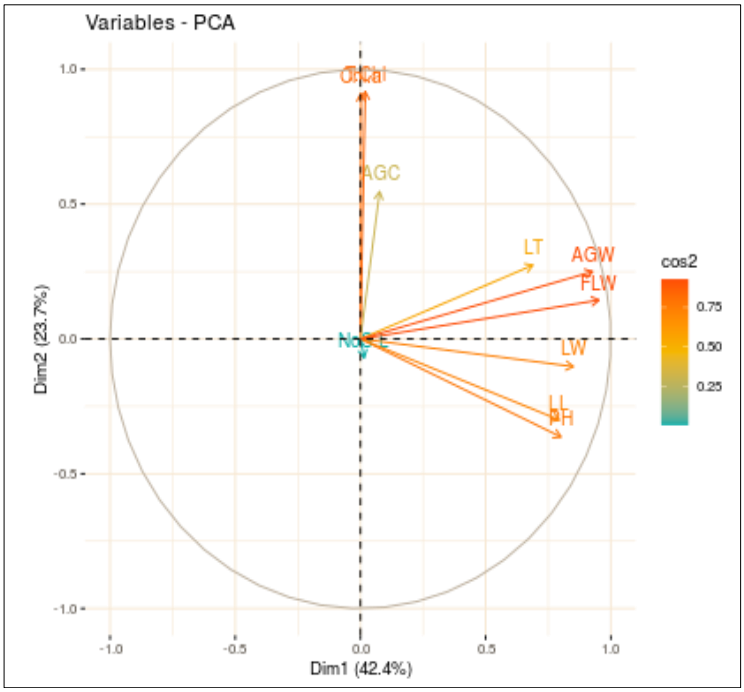

Fig 3: Contribution of variables toward principal components

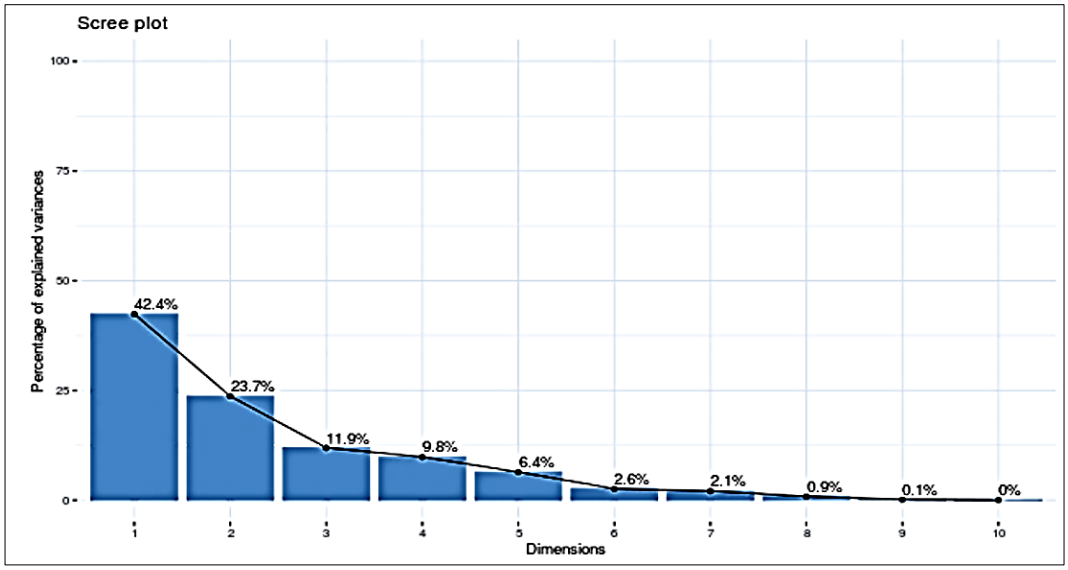

Fig 4: Screen plot diagram of percent of explained variance by principal component

Table 6: Principal component analysis for 10 traits of 37 genotypes of aloe vera

S. No.	Trait	PC-I	PC-II	PC-III
1	Plant height (cm)	0.389	-0.236	0.245
2	Leaf length (cm)	0.386	-0.196	0.249
3	Leaf width (cm)	0.413	-0.067	-0.021
4	Leaf thickness (cm)	0.334	0.178	-0.225
5	Total chlorophyll (mg/g)	0.001	0.592	0.332
6	Fresh leaf weight (g)	0.009	0.596	0.318
7	Chlorophyll-a (mg/g)	0.462	0.094	-0.049
8	No. Of spine/leaf	0.037	0.355	-0.518
9	Aloe gel content (%)	0.007	-0.046	0.565
10	Aloe gel weight (g)	0.45	0.162	-0.163

Conclusion

In present study Cluster analysis revealed that the maximum inter cluster distance was observed in between VI and VII (266.9) have single accession CGAV-19 and CGAV-35 respectively. This suggested that the hybridization programme involving genotype from these cluster is expected to give higher frequency of better segregates or desirable combination for development of useful genetic stocks or varieties. Three principal components with >1 eigen value exhibited 78% variability of the total among the traits studied. The principal component -I (PC-I) account highest variability (42.42%) and the trait fresh leaf weight (0.46), aloe gel weight (0.45), leaf width (0.41), leaf length (0.386), plant height (0.389) are responsible for variability present in it. Selection for such morphological trait is useful in preliminary evaluation to access genetic diversity among morphological distinct aloe vera genotype.

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