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Genetic diversity of *Cardiospermum halicacabum* L. in Kerala via d² analysis: Implications for conservation and use

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Abstract

A comprehensive evaluation of 68 accessions of *Cardiospermum halicacabum* L. was undertaken to assess genetic variability, trait associations, and potential for crop improvement. These accessions, conserved at the Field Gene Bank of Christian College, Kattakada, were analyzed for six agrobotanic traits, including plant height, number of branches per plant, leaf length, leaf area, biomass yield, and phytochemical content. Multivariate analysis of 60 accessions was carried out using Mahalanobis D² statistics (Mahalanobis, 1936) to quantify genetic diversity and explore interrelationships among traits. Based on D² values, clustering grouped the genotypes into 12 distinct clusters, revealing substantial genetic divergence. Clusters VIII and II exhibited the highest intercluster distance, indicating their suitability for hybridization and the potential to exploit heterosis. Cluster VII showed superior performance in leaf length and leaf area, suggesting its value for enhancing vegetative traits. Cluster XII recorded the highest biomass yield, making it a promising candidate for improving agronomic productivity. Notably, Cluster XII demonstrated elevated levels of apigenin and gallic acid, highlighting its medicinal significance and potential for phytochemical enrichment. These findings underscore the genetic richness and trait diversity within *C. halicacabum*, offering a robust framework for the selection of elite genotypes. By integrating variability metrics, trait correlations, and cluster analysis, the study strategically supports breeding programs to enhance morphological vigour and therapeutic potential, while laying a robust foundation for targeted genetic improvement, conservation, and sustainable utilization of underutilized medicinal plant resources.

Keywords: *Cardiospermum halicacabum*, Mahalanobis D² analysis, agrobotanic traits, cluster analysis, genetic diversity

Introduction

Cardiospermum halicacabum Linn. (Sapindaceae), commonly known as Balloon vine, is referred to as Karnasphota in Ayurveda and Uzhinja in Malayalam. *Cardiospermum halicacabum* exhibits trifoliolate leaves, slender pubescent stems, and characteristic inflated, bladder like capsules. In Indian traditional system Ayurveda, it is the core species for the treatment of rheumatism and lumbago (Azamthulla and Shree, 2019) ^[3]. In Unani, the seeds are mentioned as tonic and also for the treatment of cancer (Ahmad and Malik, 2010) ^[2]. This species is also used in other systems of medicines such as Siddha and Homeopathy due to its immense therapeutic nature (Bhagat and Bhuktar, 2020) ^[5]. *C. halicacabum* is reported to possess a wide range of pharmacological properties, including diaphoretic, diuretic, emetic, laxative, refrigerant, stomachic, and sudorific actions (Raman *et al.*, 1998) ^[12], antidiarrheal potential (Rao *et al.*, 2006) ^[13], antioxidant activity (Kumaran & Karunakaran, 2006), and the ability to suppress TNF production (Babu *et al.*, 2006) ^[4]. Additionally, it exhibits anticancer (Sheeba & Asha, 2006) and vasodepressant effects (Gopalakrishnan *et al.*, 1976) ^[7], antiviral activity, antiulcer activity, antidiabetic activity, anticonvulsant activity, antipyretic activity, anxiolytic activity, antiarthritic activity, antifungal activity, antiparasitic activity, antifilarial activity, antiinflammatory activity, antimalarial and antibacterial activity (Suresh and Paramakrishnan, 2023) ^[16]. The National Medicinal Plants Board (NMPB) facilitates scientific validation of ethnomedicinal claims, encouraging phytochemical and pharmacological studies that have identified bioactive compounds such as flavonoids, alkaloids, terpenoids, saponins,

and glycosides (NMPB, 2025). *C. halicacabum* is widely valued for its therapeutic uses in India and globally. Internationally, it features in creams, drops, and herbal supplements sold by Schwabe Germany, Boiron USA, and Lehning France, highlighting its global medicinal relevance and potential for expanded therapeutic use. In India, ZYREX Extract Tablets are marketed as Ayurvedic supplements for wellness and anti-inflammatory support, while SBL Ointment is used in homeopathy for eczema and psoriasis. Brands like Schwabe India, SBL, and B Jain offer tinctures and dilutions for rheumatism, joint pain, and skin inflammation. In Kerala, Vaidyaratnam uses it in tailams for joint and scalp care (Varier *et al.*, 2023)^[17].

The National Medicinal Plants Board (NMPB), under the Ministry of AYUSH, promotes cultivation of *C. halicacabum* through schemes supporting farmers and research institutions, emphasizing sustainable agrotechniques and biodiversity conservation. It is among 59 high trade, self-grown species sourced from natural habitats. Overcollection to meet rising demand is causing population decline (Goraya and Ved, 2017)^[8].

Mahalanobis D² analysis has proven to be a powerful statistical tool for assessing genetic divergence among accessions of a single species. In coriander (*Coriandrum sativum* L.), a study involving 28 ecotypes from various Indian provinces grouped genotypes into five distinct clusters, with the highest inter-cluster distance observed between clusters IV and V ($D^2 = 471.83$), suggesting potential for hybridization to exploit genetic variability (Acharya and Singh, 2021)^[1]. Similarly, in tropical carrot (*Daucus carota* L.), 80 accessions were evaluated and clustered into seven groups, with yield traits contributing most significantly to genetic diversity (38.04%) (Manisha *et al.*, 2022)

^[10]. In okra (*Abelmoschus esculentus* L.), 30 genotypes were analyzed and grouped into six clusters, where traits like branches per plant and fruit yield were key contributors to divergence (Singh *et al.*, 2018)^[15]. These studies underscore the utility of D² analysis in identifying genetically diverse parents for breeding programs aimed at improving crop performance. *Cardiospermum halicacabum*, a climber of notable medicinal value found across Kerala's diverse ecosystems, faces increasing threats that necessitate a strategic and scientific conservation framework. In this backdrop 68 accessions of the species collected from diverse ecogeographical regions of Kerala and maintained in uniform environmental conditions were analyzed using six agrobotanical traits to identify suitable parents for hybridization and clarify genetic relationships.

Materials and Methods

Field surveys across Kerala yielded 68 accessions of *Cardiospermum halicacabum*, conserved at Christian College, Kattakada (Table 1). Characterization of the accessions considering six agrobotanical traits—including leaf length, leaf breadth, leaf area, biomass yield, apigenin, and gallic acid were analyzed. Apigenin and gallic acid contents were estimated via HPLC. Multivariate analysis of 68 accessions using Mahalanobis D² statistics assessed genetic diversity and trait variation. Cluster means for each of the six agrobotanic traits were computed to characterize the distinct features of the accessions grouped within each cluster (Table 2). Inter- and intra-cluster distances were calculated to assess the degree of genetic divergence among the clusters (Table 3). Cluster Means of the six agrobotanic characters in respect of the clusters of the accessions of *Cardiospermum halicacabum* (D² analysis) (Table 4).

Table 1: Details of Collection localities of the 68 accessions of *Cardiospermum halicacabum*.

Acc. No.	Place of collection	District	Latitude	Longitude	Altitude (m)
Ch 1	Kovalam	Trivandrum	8.38°N	76.99°E	41
Ch 2	Palayam	Trivandrum	8.50°N	76.94°E	43
Ch 3	Nedumangad	Trivandrum	8.60°N	77.00°E	81
Ch 4	Thirumala	Trivandrum	8.50°N	76.99°E	63
Ch 5	Ponmudi	Trivandrum	8.77°N	77.11°E	1005
Ch 6	Kuttikkadu	Kollam	8.84°N	76.92°E	158
Ch 7	Kottarakkara	Kollam	9.01°N	76.78°E	63
Ch 8	Punalur	Kollam	9.00°N	76.92°E	103
Ch 9	Munroe Island	Kollam	8.99°N	76.61°E	23
Ch 10	Thangassery	Kollam	8.88°N	76.58°E	18
Ch 11	Adoor	Pathanamtitta	9.15°N	76.74°E	73
Ch 12	Konni	Pathanamtitta	9.21°N	76.84°E	81
Ch 13	Mallappally	Pathanamtitta	9.44°N	76.65°E	96
Ch 14	Ranni	Pathanamtitta	9.40°N	76.79°E	112
Ch 15	Chittar	Pathanamtitta	9.32°N	76.92°E	218
Ch 16	Chunakkara	Alappuzha	9.18°N	76.60°E	36
Ch 17	Karuvatta	Alappuzha	9.31°N	76.41°E	24
Ch 18	Ambalapuzha	Alappuzha	9.37°N	76.35°E	20
Ch 19	Mararikulam	Alappuzha	9.60°N	76.31°E	29
Ch 20	Cherthala	Alappuzha	9.68°N	76.34°E	29
Ch 21	Ettumanoor	Kottayam	9.65°N	76.54°E	59
Ch 22	Kondoor	Kottayam	9.70°N	76.75°E	53
Ch 23	Vadavathoor	Kottayam	9.59°N	76.56°E	40
Ch 24	Kanjirappally	Kottayam	9.55°N	76.78°E	113
Ch 25	Kumarakom	Kottayam	9.61°N	76.43°E	21
Ch 26	Muvattupuzha	Ernakulam	9.97°N	76.58°E	52
Ch 27	Aluva	Ernakulam	10.11°N	76.37°E	33
Ch 28	Thevara	Ernakulam	9.93°N	76.29°E	22
Ch 29	Malayattoor	Ernakulam	10.20°N	76.49°E	59
Ch 30	Peermade	Idukki	9.59°N	76.96°E	1002

Ch 31	Adimali	Idukki	10.00°N	76.96°E	613
Ch 32	Idukki Twp	Idukki	9.85°N	76.95°E	830
Ch 33	Anavilasam	Idukki	9.65°N	77.10°E	1104
Ch 34	Vandiperiyar	Idukki	9.57°N	77.08°E	852
Ch 35	Pananchery	Thrissur	10.55°N	76.31°E	54
Ch 36	Edakkara	Thrissur	10.64°N	75.99°E	21
Ch 37	Kottappuram	Thrissur	10.67°N	76.20°E	58
Ch 38	Kolazhy	Thrissur	10.56°N	76.21°E	20
Ch 39	Pullur	Thrissur	10.34°N	76.23°E	26
Ch 40	Panangattiri	Palakkad	10.60°N	76.67°E	106
Ch 41	Pathirippala	Palakkad	10.78°N	76.48°E	96
Ch 42	Kinassery	Palakkad	10.72°N	76.67°E	111
Ch 43	Mannarkad-i	Palakkad	10.97°N	76.47°E	108
Ch 44	Kulakkad	Palakkad	10.88°N	76.36°E	83
Ch 45	Kodumudi	Malappuram	10.87°N	76.11°E	38
Ch 46	Varangode	Malappuram	11.05°N	76.07°E	33
Ch 47	Pandalur	Malappuram	11.08°N	76.17°E	56
Ch 48	Mampad	Malappuram	11.25°N	76.19°E	53
Ch 49	Areekode	Malappuram	11.23°N	76.04°E	86
Ch 50	Triprangode	Malappuram	10.84°N	75.94°E	29
Ch 51	Thamarassery	Kozhikode	11.42°N	75.94°E	68
Ch 52	Kuttiady	Kozhikode	11.66°N	75.73°E	35
Ch 53	Iringal	Kozhikode	11.52°N	75.61°E	30
Ch 54	Chemancheri	Kozhikode	11.40°N	75.71°E	19
Ch 55	Azhinjilam	Kozhikode	11.20°N	75.86°E	58
Ch 56	Madathumpadi	Wayanad	11.64°N	76.08°E	817
Ch 57	Kidanganad	Wayanad	11.67°N	76.25°E	856
Ch 58	Irulam	Wayanad	11.76°N	76.19°E	890
Ch 59	Pulpally	Wayanad	11.80°N	76.06°E	748
Ch 60	Thavinhal	Wayanad	11.82°N	75.97°E	785
Ch 61	Valiyannur	Kannur	11.89°N	75.41°E	59
Ch 62	Nuchiyad	Kannur	12.03°N	75.60°E	61
Ch 63	Taliparamba	Kannur	12.05°N	75.37°E	92
Ch 64	Pazhayangadi	Kannur	12.03°N	75.26°E	57
Ch 65	Muzhappilangad	Kannur	11.79°N	75.45°E	26
Ch 66	Kallar	Kasargod	12.43°N	75.26°E	160
Ch 67	Kakkat	Kasargod	12.28°N	75.14°E	55
Ch 68	Udma	Kasargod	12.43°N	75.02°E	44

Table 2: Composition of the clusters of the accessions of *Cardiospermum halicacabum* based on D² analysis

Cluster	No. Of Accessions	Accessions Code
I	11	Ch 8, Ch 10, Ch 17, Ch 19, Ch 20, Ch 26, Ch 35, Ch 41, Ch 61, Ch 62, Ch 63
II	9	Ch 1, Ch 3, Ch 6, Ch 11, Ch 21, Ch 22, Ch 25, Ch 43, Ch 48
III	2	Ch 13, Ch 50
IV	4	Ch 2, Ch 24, Ch 36, Ch 42
V	7	Ch 46, Ch 49, Ch 51, Ch 53, Ch 58, Ch 60, Ch 65
VI	5	Ch 9, Ch 15, Ch 28, Ch 57, Ch 64
VII	9	Ch 5, Ch 23, Ch 29, Ch 45, Ch 47, Ch 52, Ch 56, Ch 59, Ch 66
VIII	4	Ch 18, Ch 30, Ch 38, Ch 54
IX	7	Ch 16, Ch 31, Ch 32, Ch 37, Ch 40, Ch 55, Ch 67
X	4	Ch 4, Ch 12, Ch 14, Ch 33
XI	5	Ch 7, Ch 27, Ch 34, Ch 39, Ch 44
XII	1	Ch 68

Table 3: Inter- and intracluster distance of the clusters of the accessions of *Cardiospermum halicacabum* (D² analysis)

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
I	4.00133											
II	23.70808	4.78017										
III	16.44350	8.10507	4.63772									
IV	7.39434	28.60155	20.72151	3.92470								
V	6.28873	29.58491	22.43816	7.08007	1.10614							
VI	7.81481	21.86690	13.91112	8.55047	12.73460	2.87042						
VII	6.32791	29.57594	21.96097	4.20114	3.84740	10.49849	2.48154					
VIII	7.34514	30.76546	23.29589	6.16996	4.67183	11.49732	3.38464	1.79163				
IX	18.73314	6.55899	6.84946	24.57160	24.41366	18.58310	24.80742	25.92480	2.68857			
X	13.75238	10.55065	6.06374	19.69502	19.48405	14.14488	19.86186	20.92251	5.08681	4.34975		
XI	3.75072	20.15400	13.14052	10.24753	9.49705	8.15982	9.87171	11.06418	15.11563	10.13882	5.29049	
XII	12.74168	14.36838	10.85192	19.60454	17.67275	15.67388	18.91806	19.66178	8.21865	4.95094	9.39206	4.10236

Table 4: Means of the six agrobotanic characters in respect of the clusters of the accessions of *Cardiospermum halicacabum* (D² analysis)

Sl. No.	Characters (Mean)	Clusters											
		I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
1	Leaf Length	6.591	7.346	7.833	7.028	7.457	8.127	8.345	8.335	7.530	6.193	6.070	6.230
2	Leaf Breadth	4.557	5.752	5.771	5.151	5.540	6.005	6.027	5.985	5.470	4.103	3.730	4.670
3	Leaf Area	19.512	27.474	29.386	23.558	26.833	31.743	32.572	32.390	26.740	16.500	14.720	18.920
4	Biomass yield	9.47	11.733	14.515	14.17	11.442	13.642	10.45	11.975	14.095	10.347	11.054	17.29
5	Apigenin	0.005	0.014	0.004	0.011	0.005	0.011	0.006	0.006	0.005	0.015	0.013	0.024
6	Gallic Acid	0.026	0.029	0.048	0.018	0.047	0.041	0.026	0.017	0.021	0.018	0.047	0.086

Results and Discussion

The present investigation employed Mahalanobis D² analysis to assess genetic divergence among 68 accessions of *Cardiospermum halicacabum* collected from diverse ecogeographical regions of Kerala, evaluated across six agrobotanical traits. This multivariate approach has proven instrumental in identifying genetically distinct parents for hybridization programs in several medicinal and crop species, as demonstrated in coriander, tropical carrot, and okra (Acharya and Singh, 2021; Manisha *et al.*, 2022; Singh *et al.*, 2018) ^[1, 10, 15].

The 68 accessions were grouped into twelve distinct clusters, indicating substantial genetic diversity within the species across Kerala's varied ecosystems (Table 2). The distribution pattern revealed considerable variation, with Cluster I being the largest (11 accessions), followed by Clusters II and VII (9 accessions each), while Cluster XII contained a solitary accession (Ch 68 from Udma, Kasargod). The formation of a single-member cluster suggests that this accession possesses unique genetic characteristics markedly different from all other accessions, warranting special attention for conservation and potential breeding applications.

The clustering pattern did not strictly follow geographical proximity, suggesting that factors beyond geographical isolation such as microclimatic conditions, soil types, and altitude may influence genetic differentiation in *C. halicacabum*. For instance, accessions from the same district were distributed across multiple clusters, indicating substantial intra-regional genetic variation. This observation aligns with findings in other medicinal plant species where environmental heterogeneity contributes significantly to genetic diversification (Christopher *et al.*, 2018) ^[6].

Analysis of inter-cluster distances revealed maximum genetic divergence between Clusters II and VIII (D² = 30.76546), followed by Clusters II and V (D² = 29.58491) and Clusters II and VII (D² = 29.57594) (Table: 2). These substantial distances indicate that accessions from these cluster combinations represent the most genetically diverse gene pools and would be ideal candidates for hybridization programs aimed at maximizing heterosis and genetic variability. The high divergence between Cluster II, which includes accessions primarily from southern and central Kerala districts (Trivandrum, Kollam, Pathanamthitta, Kottayam, and Palakkad), and Cluster VIII, comprising accessions from Alappuzha, Idukki, Thrissur, and Kozhikode, suggests that geographical and ecological factors have contributed to significant genetic differentiation.

Conversely, the minimum inter-cluster distance was observed between Clusters V and VII (D² = 3.84740), indicating relatively closer genetic relationships. Despite this proximity, both clusters maintained distinct identities, suggesting subtle but consistent differences in their genetic makeup. Intra-cluster distances ranged from 1.10614 (Cluster V) to 5.29049 (Cluster XI), with lower values indicating homogeneity within clusters. The

relatively low intra-cluster distance in Cluster V suggests that accessions within this group share similar genetic backgrounds, while the higher value in Cluster XI indicates greater within-cluster variation.

Examination of cluster means (Table: 4) for the six agrobotanical characters revealed distinct patterns contributing to genetic divergence. Leaf morphometric traits—leaf length, breadth, and area showed considerable variation across clusters. Clusters VII and VIII exhibited the highest mean values for leaf length (8.345 cm and 8.335 cm, respectively) and leaf area (32.572 cm² and 32.390 cm²), while Cluster XI displayed the lowest values (6.070 cm length, 3.730 cm breadth, and 14.720 cm² area). These morphological variations likely reflect adaptive responses to diverse environmental conditions across Kerala's ecological zones, from coastal plains to high-altitude regions.

Biomass yield demonstrated substantial variation, with Cluster XII (the single-member cluster) exhibiting the highest mean (17.29 g), followed by Cluster III (14.515 g) and Cluster IX (14.095 g). This exceptional biomass yield in Ch 68 further underscores its uniqueness and potential value for crop improvement programs. The lowest biomass yield was recorded in Cluster I (9.47 g), suggesting that accessions in this cluster may be adapted to resource-limited environments or have allocated resources preferentially to other fitness-related traits.

Phytochemical analysis revealed notable variations in apigenin and gallic acid contents across clusters. Apigenin content was highest in Cluster XII (0.024%), followed by Clusters X (0.015%) and II (0.014%), while Cluster III showed the lowest concentration (0.004%). Similarly, gallic acid content peaked in Cluster XII (0.086%), with Cluster III also showing elevated levels (0.048%), while Clusters IV, X, and VIII exhibited lower concentrations (0.018%, 0.018%, and 0.017%, respectively). These variations in bioactive compound concentrations are particularly significant given the therapeutic applications of *C. halicacabum* in treating rheumatism, inflammation, and various other ailments. The exceptional phytochemical profile of Ch 68 (Cluster XII) positions it as a valuable genetic resource for developing high-yielding chemotypes with enhanced medicinal properties.

The extensive genetic diversity revealed by this study has important implications for both conservation strategies and breeding programmes. The identification of genetically divergent clusters, particularly the maximum divergence between Clusters II and VIII, provides a scientific basis for selecting parents in hybridization programmes. Crosses between accessions from these divergent clusters are expected to generate progeny with maximum heterosis and broad genetic variability, facilitating selection for desirable traits such as high biomass yield and elevated phytochemical content.

The unique status of Ch 68 (Cluster XII) from Udma, Kasargod, with its superior biomass yield and exceptional phytochemical content, makes it a priority accession for conservation and utilization in breeding programmes. Its combination of high apigenin and gallic acid content with substantial biomass

production represents an ideal chemotype for pharmaceutical applications. Similarly, accessions from Clusters II, III, and X, which showed relatively high phytochemical content, warrant preservation and further investigation.

The distribution of genetic diversity across Kerala's districts highlights the importance of *in situ* conservation efforts spanning the state's diverse ecological zones. The lack of strict geographical clustering suggests that genetic diversity is maintained through gene flow and local adaptation to microenvironmental conditions. This underscores the need for conservation strategies that protect accessions from multiple geographical regions rather than focusing on a single biodiversity hotspot.

The patterns of genetic divergence observed in *C. halicacabum* are comparable to those reported in other medicinal and crop species subjected to D² analysis. In *Bacopa monnieri*, the maximum inter-cluster distance (D² = 3378.6) was substantially higher than that observed in our study, possibly reflecting greater morphological and ecological diversity in that species (Christopher *et al.*, 2018)^[6]. However, the clustering pattern and the utility of D² analysis in identifying suitable parents for hybridization remain consistent across species. Similar to findings in tropical carrot, where yield traits contributed significantly to genetic diversity (38.04%), biomass yield and phytochemical content emerged as important contributors to divergence in *C. halicacabum* (Manisha and Padmini, 2022)^[10]. Given that *C. halicacabum* is among 59 high-trade species facing population decline due to overcollection from natural habitats, the genetic diversity documented in this study provides crucial baseline information for developing conservation action plans. The National Medicinal Plants Board's initiatives to promote cultivation through sustainable agrotechniques must be informed by genetic diversity assessments to ensure that cultivated populations maintain adequate genetic variation for long-term viability.

The establishment of core collections representing the genetic diversity captured in this study would facilitate both conservation and utilization objectives. Priority should be given to preserving accessions from genetically distinct clusters, particularly those with unique phytochemical profiles or adaptation to specific ecological conditions. *Ex situ* conservation in field gene banks, complemented by *in situ* conservation of wild populations, would provide insurance against genetic erosion while maintaining evolutionary processes.

This study establishes a foundation for future research on *C. halicacabum* genetics and breeding. Molecular marker-based studies could complement morphological and phytochemical characterization, providing insights into genomic diversity and population structure. Controlled hybridization experiments between genetically divergent clusters would validate the breeding potential suggested by D² analysis and facilitate the development of improved varieties with enhanced medicinal properties.

Further investigation into the environmental factors driving genetic differentiation would inform adaptive management strategies and help predict how populations might respond to climate change. Since the species is present across Kerala's diverse altitudinal gradient (18 to 1104 m), studies on adaptation to altitude and associated climatic variables could reveal valuable insights into stress tolerance mechanisms.

Conclusion

The Mahalanobis D² analysis successfully revealed substantial genetic diversity among *C. halicacabum* accessions from Kerala,

with the formation of twelve distinct clusters and identification of genetically divergent groups suitable for hybridization. The exceptional characteristics of Ch 68 from Kasargod, combined with the overall diversity pattern, provide a strong scientific basis for conservation prioritization and breeding programme development. These findings contribute to the broader objective of ensuring sustainable utilization of this valuable medicinal plant species while safeguarding its genetic resources for future generations.

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