Research Article

Int J Pharma. Bio. Sci, Vol: 17, Issue: 1, 2026; 6-11

International Journal of Pharma and Biosciences

Content Available at www.lapinjournals.com ISSN: 0975-6299

COMPARATIVE KARYOMORPHOLOGICAL ANALYSIS OF IN VIVO GROWN PLANT SPECIES OF *ALOE* FROM DIFFERENT REGIONS OF INDIA

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Article History: Received: 25.Sept.2025 Revised: 14.Nov.2025 Accepted: 30.Jan.2026

Abstract

Comparing the karyomorphological characteristics of five different *Aloe vera* species that were collected from different regions of India and cultivated in vivo is the aim of this study. Cytological investigations were conducted to evaluate the distinctions among these species. The centromeric index, chromosome number, karyotype asymmetry, and total chromatin length were measured in order to detect variations. The data revealed significant inter-varietal variations, indicating that these *Aloe* species can differ from one another cytogenetically. Variations in chromosomal structure, centromeric position, and chromatin organization were found using karyotypic analysis. Cytogenetic traits and chromosomal variations were to be evaluated in order to understand the evolutionary links and genetic diversity among these species. This study provides valuable information about the chromosomal architecture and evolutionary cytogenetics of *Aloe* species in India, while also emphasizing the importance of karyological data in supporting species classification, biodiversity conservation, and the selection of superior genotypes for cultivation and pharmacological research.

Keywords: *Aloe vera*, karyotype, chromosome morphology, ideogram, genetic diversity.

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DOI: https://doi.org/10.22376/ijpbs.v17i1.138

INTRODUCTION

As a tropical perennial succulent plant belonging to the Asphodelaceae family, *Aloe vera* thrives in hot, arid climates.[1]. Renowned for its industrial, therapeutic, and medical applications [2, 3]. Its anti-inflammatory, wound-healing, and immunomodulatory qualities are believed to be caused by a variety of bioactive substances, such as polysaccharides, anthraquinones, and vitamins [4, 5]. India's diverse agroclimatic conditions have led to the growth of numerous *Aloe vera* cultivars, each with unique morphological and biochemical characteristics [6]. *Aloe vera* is a plant that originated in southern Africa and is now grown all over the world [7].It can be found in Andhra Pradesh. For millennia, people have acknowledged and used its health, cosmetic, medicinal, and skin care benefits [8]. It has grown in significance as a result of its several therapeutic advantages [9]The remaining 75% of the plant, which is primarily made up of water, is made up of sugar, enzymes, vitamins, minerals, lignin, tannic acid, polysaccharides, glycoproteins, saponins, sterols, amino acids, and salicylic acid ([10].Cytogenetic studies are crucial for understanding genetic diversity, species differentiation, and evolutionary processes [11].Karyomorphological study, which looks at chromosomal number, structure, and asymmetry, can help us identify the genetic relationships of *Aloe* species [12].Due to its well-known medicinal, cosmetic, and pharmaceutical applications, aloe vera is grown in many different varieties throughout different regions [13].Cytogenetic studies are crucial for understanding genetic diversity, species differentiation, and evolutionary processes [11]. Karyomorphological study, which looks at chromosomal number, structure, and asymmetry, can help us identify the genetic relationships of *Aloe* species [12].This study's primary focus is on the karyotypic variations among five *Aloe* vera species that were collected from different parts of India. Despite considerable efforts to explore the phytochemical and therapeutic potential of *Aloe* species, cytological and karyomorphological studies remain limited [4, 26]. By considering chromosomal number, morphology, and asymmetry indices, karyotype analysis provides a better understanding of plant species taxonomic position, evolutionary relationships, and genetic divergence [14].This kind of

cytogenetic knowledge is essential for species identification, germplasm conservation, and understanding the mechanisms of chromosomal evolution in angiosperms. *Aloe barbadensis Miller* has a diploid chromosomal number of 2n = 14, with chromosomes that are mainly metacentric to submetacentric, according to previous studies [15].However, comprehensive comparative cytogenetic studies involving lesser-known species like *A. perryi*, *A. Cim-Sheetal*, and *A. trinervis* are scarce. Because these species have adapted to the many environmental conditions prevalent in India, they may have undergone karyotypic modifications that reflect their evolutionary histories. A comparative karyomorphological analysis of five aloe species collected from different regions of India is the goal of the current study. By evaluating chromosomal traits like arm ratio, centromere position, total chromosome length, and asymmetry indices, we hope to identify cytogenetic relationships and further our understanding of chromosomal evolution within the genus *Aloe*.

MATERIALS AND METHODS

Plant material collection

Four species and one variety were chosen for this study. The Central Institute of Medicinal and Aromatic Plants (CIMAP) in Lucknow provided Aloe perryi and *Aloe Cim-sheetal*, while the University of Science and Technology Meghalaya (USTM) in Meghalaya provided

S NO.	Name	Place of collection
1	*Aloe perryi*	CIMAP, Lucknow, UP
2	*Aloe Cim-sheetal*	CIMAP, Lucknow, UP
3	*Aloe barbadensis Miller*	USTM (University of Science and Technology, Meghalaya)
4	*Aloe vera*	Central University, Ranchi
5	*Aloe trinervis*	NBRI (Nationl Botanical Research Institute), Lucknow, UP

Aloe barbadensis miller. *Aloe vera* is sourced from the Central University of Jharkhand (CUJ) in Ranchi, whereas *Aloe trinervis* is sourced from the National Botanical Research Institute (NBRI) in Lucknow. Mature plants free of disease were chosen for cytological examination. For cytological investigations, plant samples of *Aloe perryi*, *Aloe Cim-Sheetal*, *Aloe barbadensis*Miller, *Aloe vera*, *and Aloe trinervis* were gathered from their locations and kept under open field cultivation settings [16].

Root tip preparation for cytology study

To stop cells in metaphase, actively growing root tips were gathered and pre-treated for three to four hours at room temperature in a saturated aqueous solution of 8-hydroxyquinoline (0.002 M) [15].The pre-treated root tips were stored in 70% ethanol at 4°C for later usage after being fixed in Carnoy's solution (3:1 ethanol: acetic acid) for 24 hours. They carried out cytological studies [17].

Staining and slide preparation

After 10 minutes of hydrolysis in 1N HCl at 60°C, the fixed root tips were stained with Feulgen reagent, which produces a distinct chromosomal contrast, for an hour in the dark [18].To obtain well-spread metaphase chromosomes, the meristematic area was gently pressed with a cover slip to create squash preparations.

Feulgen stain was applied after the fixed root tips which produces a distinct chromosomal contrast, for an hour in the dark[18],had been hydrolysed in 1N HCl for ten minutes at 60°C [19] .For microscopic analysis, To obtain well-spread metaphase chromosomes, the meristematic area was gently pressed with a cover slip to create squash preparations.squash preparations were prepared with 45% acetic acid [20].

Microscopy and karyotype analysis

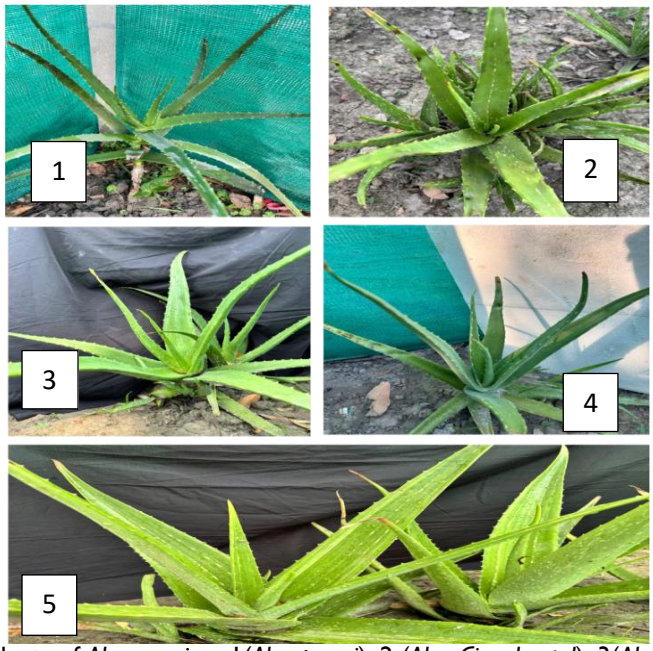
A Temp star XCAM 1080PHB/PHD HDMI light microscope was used to view the prepared slides at 40x and 100x magnification. in order to distinguish between species, and ideograms were created. To perform statistical analysis, SPSS 10 and ImageJ was used to in order to distinguish between species, and ideograms were created. About 1000 cells were examined in which 10 evenly distributed metaphase cells per species .order to evaluate the quantity, size, and shape of the chromosomes the total chromatin length, chromosome number, centromeric index, and karyotype asymmetry [12]. Chromosomes were classified into metacentric, submetacentric, and sub telocentric types accordingto centromere position. The karyomorphological parameters listed below were noted:

Statistical analysis

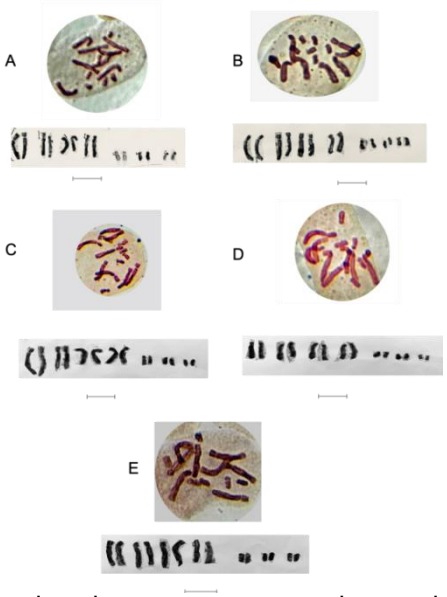
To determine the significance of differences in chromosome length, arm ratio, and asymmetry indices between species, the data were put through a one-way ANOVA. karyotype parameters were performed using **SPSS10** to explore relationships among species [21].

Table 01" Details of *Aloe* species and their respective place of collection.

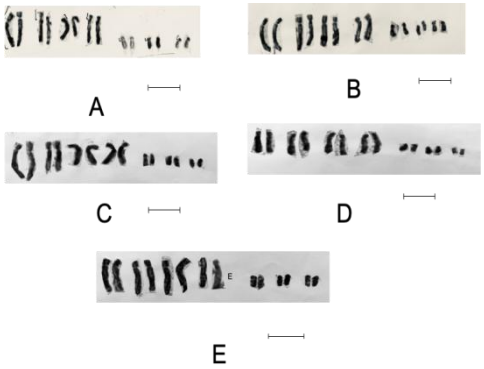
Parameter
Chromosomes number
Total haploid chromosomes length (THL)
Longest chromosomes length
Shortest chromosomes length
Arm ratio (long arm/short arm)
Centromeric index
Karyotypic formula and chromosomes types



Figs 01-1-5: In vivo grown plants of *Aloe* species- 1(*Aloe perryi*), 2 (*Aloe Cim-sheetal*), 3(*Aloe barbadensismiller*), 4(*Aloe vera*), 5(*Aloe trinervis)*



Figs 02: A-E.Karyogram and corresponding ideogram representing the complete chromosomal complement. The karyogram displays metaphase chromosomes arranged by size and centromere position, while the ideogram provides a schematic representation of individual chromosomes with banding patterns and structural features. Scale bars = 100µm.



Figs 03: A-E.Ideogram of *A. perryi* (A), *A.Cim-Sheetal* (B), *A. barbadensis Miller* (C), *A.vera* (D). *A.trinervis* (E) symbolizing the diploid chromosomes. All scale bars are 100µm.

Table 03: Estimation of genetic parameter for all the five species of *Aloe*.

Name of the species	SCN (2n)	PL (X)	KF	CP	RSCL (µm)	RLCL (µm)	TCL (µm)	TF (%)	VC
Aloe perryi	14	2x	4m+6Sm+2St+2Sm(SAT)	1	1.8-3.9	3.7-9.3	9.62	33.17	33.29
Aloe Cim-Sheetal	14	2x	8St+6sm	-	1.8-2.7	3.8-8.9	8.53	33.18	33.30
Aloe barbadensis Miller	14	2x	2St(SAT)+6St+6Sm	1	1.6-3.5	2.2-7.5	7.31	34.77	33.52
Aloe vera	14	2x	6Sm+8St(2SAT)	1	1.2-3.0	1.6-3.5	4.93	46.09	25.35
Aloe trinervis	14	2x	8St+6Sm	-	1.4-4.2	2.5-9.5	58.91	31.76	32.94

Abbreviations:SCN somatic chromosomes number; PL ploidy level; KF karyotypic formula; CP chromosomes pairs with secondary constriction; RSCL range of short arm length; RLCL range of long arm length; TCL total chromatin length in (µm); VC variation coefficient in percentage ; TF total forma percentage;

Table 04:morphological details of chromosomes from haploid karyotype of all five aloe species.

Name of the species	Chromosomes group	Total length(µm)	Short arm(µm)	Centromeric index(%)	Arm ratio (%)	Centromeric position	Secondary constriction
Aloe perryi	A	9.4	2.7	30.41	2.1	Sm	present
Aloe Cim-Sheetal	A	8.5	2.8	34.46	1.9	St	absent
Aloe barbadensis Miller	A	7.3	2.5	36.07	1.8	St	present
Aloe vera	A	4.9	2.2	46.09	1.1	Sm	present
Aloe trinervis	A	8.5	2.6	31.81	2.1	St	absent

RESULTS AND DISCUSSION

In vivo grown plants of five different species of aloe were subjected to a comparative diploid karyotype analysis. Comprehensive numerical data analysis revealed that, despite their similarities, the aloe species' karyotypes could be differentiated primarily by karyotype symmetry, satellite type and position, and mean chromosome length. Among the kinds being studied, differences in chromosome length, total chromatin length (TCL), total frequency percent (TF%), and other karyotypic characteristics were observed. All five *Aloe vera* species had a chromosome number of 2n = 14, according to karyotypic study; nevertheless, variations were noted in chromatin length, centromeric index, and chromosomal architecture [12]. A symmetrical karyotype was indicated by the mostly metacentric chromosomes of *Aloe perryi* from CSIR, Lucknow, which had a total chromatin length of 32.8 µm[11].With a chromatin length of 31.5 µm, *Aloe Cim-Sheetal* from CSIR, Lucknow, displayed a mixture of metacentric and submetacentric chromosomes [22].According to [23]. *Aloe barbadensis Miller* from USTM, Meghalaya, exhibited a chromatin length of 30.8 µm and a slightly asymmetric karyotype with submetacentric dominance. A more stable karyotype was shown by Ranchi Central University aloe vera's larger fraction of metacentric chromosomes and chromatin length of 33.2 µm[16]. *Aloe trinervis*fromNBRI, Lucknow, had acrocentric chromosomes and a chromatin length of 29.6 µm, indicating a greater asymmetry index [24].

Karyotype analysis of *Aloe perryi*

A. perryi has 2n=2x=14 (8L+6S) diploid chromosomes (fig. 3 (A)). The karyotype was asymmetrical and bimodal, with three short (S) and four long (L) pairs of chromosomes. The long chromosomes were between 3.7 and 9.3 µm long, whereas the short ones were between 1.8 and 3.9 µm long. 9.62µm was the overall chromatin length, and 33.17 percent of the total forms were found. The karotypic formula was 2n=2x=14=4m+6Sm+2St+2Sm (SAT), which includes two subtelocentric chromosome segments, eight submetacentric chromosome regions, and four metacentric regions.On the long arm of chromosomes of the diploid karyotype, one pair of satellite and secondary constriction were seen.

Karyotype analysis of *Aloe Cim-Sheetal*

*A. Cim-Sheetal*somatic chromosomal count was 2n=2x=14 (8L+6S) (fig. 3 (B)). The karyotype was asymmetrical and bimodal, with three short (S) and four long (L) pairs of chromosomes. The long and short chromosomes have lengths ranging from 3.8 to 8.9 µm and 1.8 to 2.7 µm, respectively. 8.53µm was the overall chromatin length, and 33.18 percent of the total forms were found. The chromosomes' karotypic formula was 2n=2x=14=8St+6Sm, which includes 8 subtelocentric and 6 submetacentric regions. On both the long and short arms of the diploid karyotype's chromosomes, no satellite or secondary constriction was observed.

Karyotype analysis of *Aloe barbadensis Miller*

Figure 3 (C) shows that *A. barbadensis Miller* had 2n=2x=14 (8L+6S) diploid chromosomes. The karyotype was asymmetrical and bimodal, with three short (S) and four long (L) pairs of chromosomes. The long and short chromosomes have lengths ranging from 2.2 to 7.5 µm and 1.6 to 3.5 µm, respectively. 7.31µm was the overall chromatin length, and 34.77 percent of the total forms were found. The karyotypic formula was 2n=2x=14=2St(SAT)+6St+6Sm (), which

includes 6 sub median chromosome regions and 8 sub telocentric regions. One pair of satellite and secondary constriction was seen in the long arm of long chromosomes of the diploid karyotype.

Karyotype analysis of *Aloe vera*

A.vera has 2n=2x=14 (8L+6S) somatic chromosomes (fig. 3(D)). Bimodal and asymmetrical, the karyotype has three short (S) and four long (L) pairs of chromosomes. Short chromosomes ranged in length from 1.4 to 3.0 µm, while long chromosomes ranged from 1.6 to 3.5 µm. A total of 46.09 forms were found, and the chromatin length was 4.93µm. The karyotypic formula, which contains eight sub telocentric and eight submetacentric chromosomal areas, was 2n=2x=14=6Sm+8St+2(SAT). On the long arm of long chromosomes of the diploid karyotype, two satellite and secondary constriction were seen.

Karyotype analysis of *Aloe trinervis*

A.trinervis has 2n=2x=14 (8L+6S) diploid chromosomes (fig. 3(E). The karyotype has three short (S) and four long (L) pairs of chromosomes, making it bimodal and asymmetrical. The short chromosomes ranged in length from 1.2 to 4.2 µm, while the large chromosomes ranged from 2.5 to 9.5 µm. The total forms percentage was 31.76 and the total chromatin length was 58.91µm. The karyotypic formula was 2n=2x=14=8St+6Sm, which includes 6 sub median and 8 sub telocentric chromosome regions. Secondary constriction was observed on either arm of the diploid karyotype's chromosomes, and there are no satellites.

Cytological investigations verified the previously reported somatic chromosomal number of 2n=2x=14 (8L+6S) for all five species of *Aloe*. The karyological data clearly showed that all aloe species possessed a diploid somatic bimodal asymmetric karyotype. *A. Perry* long and short chromosome arms were significantly longer than *A. Vera*. Additionally, it should be observed that the somatic chromosome complements of *A. perry*, *A. barbadensis Miller*, and *A. vera* are each coupled to the long arm of two chromosomes and one pair of secondary constrictions (Fig. 3. A, C, &D). Among other things, there were karyotypic differences between the types that were studied in terms of chromosome length, total chromatin length (TCL), coefficient variation, total frequency percentage (TF%), and karyotypic formula. The length of the longest and shortest chromosomes was determined to be 3.8-8.9 µm and 1.8-2.7 µm in *A. perryi* and 1.6-3.5 µm and 1.2-3.0 µm in *A. vera,* respectively. *A. vera* had the highest total frequency percent (46.09), whereas *A. perryi* had the lowest (33.17 µm). As a result, *A. trinervis*has a longer chromatin length (58.91µm) than *A. vera*. The erosion of chromatid components over evolution may be the cause of this decrease in chromatin length. Furthermore, the two *Aloe* species (*A. perryi*and*A. cim-Sheetal*) under study exhibit a nearly same tendency in their % coefficient of variation (CV), indicating their close kinship. (Table 2.). Higher plants with asymmetric karyotypes are thought to be more evolved than other tatas [25] . When it comes to karyotypic orthoselection—the homogeneity of basic chromosomal number and morphology-Aloaceae is one of the most stable angiogram families. The vast genus *Aloe* lies among the primarily African family Aloaceae, which exhibits the highest degree of karyotypic orthoselection. Chromosome homogeneity exhibiting a consisting bimodal type was shown by diploid karyotype data of *Aloe* species plants cultivated in vivo. Additionally, plants that are haploid, diploid, or tetraploid exhibit widespread intra- and inter-chromosome symmetry. The research also shows that under viva circumstances, distinctive gross chromosomal morphology is preserved. The karyomorphological differences between the taxa may be resolved by more thorough molecular cytogenetic research, which would necessitate a taxonomic change.

ACKNOWLEDGEMENTS

The plant material used to complete the research was provided by CIMAP, Lucknow, USTM, Meghalaya, CUJ, Ranchi, and NBRI, Lucknow, India, for which the authors are grateful. Additionally, the authors would like to express their gratitude to Mahatma Gandhi Central University's Department of Botany Head for providing laboratory facilities.

FUNDING

No funding was received for this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

INFORMED CONSENT

This research was conducted on plant materials under laboratory conditions and did not involve human participants or animals. Therefore, informed consent was not applicable

ETHICAL STATEMENT

This research involved plant materials only and did not include human or animal subjects, hence, ethical approval was not required.

AUTHOR CONTRIBUTION

Authors: SHABNAM KUMARI (conducted the research, including conceptualization, data collection, analysis, and manuscript writing.)

Co- authors: TARA CHANDA RAM contributed by reviewing and editing the whole manuscript.

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