Asian Science Bulletin

OPEN ACCESS

Karyotype Analysis of Four Zingiberaceae Species and their Taxonomic Significance in Systematic Botany

Dozie-Akaniro Ijeoma Nkeiru, Ferdinand Nkem Mbagwu and Chibuikem Unamba
Department of Plant Science and Biotechnology, Imo State University Owerri, Nigeria

ABSTRACT

Background and Objective: Karyotype analysis among the Zingiberaceae is an important parameter in systematic botany. Karyotype analysis of four species of the family Zingiberaceae and their taxonomic relevance in systematic botany were investigated in this study. **Materials and Methods:** Herbarium specimens and living plants were collected, preserved, and identified using standard botanical protocols. Cytogenetic analysis involved mitotic chromosome preparation from root tips and detailed karyotype assessment. Measurements included chromosomal lengths, centromeric indices, and asymmetry parameters for taxonomic evaluation. **Results:** *Aframomum melegueta* has 2n = chromosomes with one metacentric, two submetacentric, and four subtelocentric chromosomes. The length of long arm chromosomes (LA) ranged from 1.56 to 3.50 µm length of short arm chromosomes (SA) ranged from 0.71 to 1.31 µm. *Curcuma longa* has 2n = chromosome with three submetacentric and four sub telocentric chromosomes. The length of long arm chromosome LA ranged from 2.5-4.48 µm. For *E. cardamomum* chromosome number was 2n = with three metacentric, three submetacentric, and one sub t elocentric chromosomes. The length of the long arm chromosome (LA) was between 1.80 and 3.77 µm, and length short arm (SA) was between 0.60 and 2.3 µm. In *Z. officinale*, chromosome numbers were 2n = with one metacentric, three submetacentric, and three subtelocentric chromosomes. The length of the long arm chromosome (LA) was between 1.73 and 3.75 µm, and the length of the short arm (SA) was between 0.51 and 2.70 µm. The Karyotype analysis of the four Zingiberaceae species studied can be used to taxonomically differentiate as well as relate them. **Conclusion:** Therefore, the confusion and controversies over the taxonomic relationship among some species in the family Zingiberaceae have been reduced, but there is still a need for more taxonomic studies and re-characterization of the species using other lines of taxonomic studies.

KEYWORDS

Karyotype analysis, Zingiberaceae, chromosome count, taxonomy, metacentric chromosomes, phytochemical constituents

INTRODUCTION

Taxonomy, the science of discovery, description, and classification of living organisms on earth, is a fundamental basis for biodiversity informatics. Taxonomists are also often involved with specimen identification. The foundations of this discipline are laid on the significant contributions of many botanists,

ISSN: 1813-0070 (Print)
https://doi.org/10.3923/asb.2025.140.145

Received: 19 Mar. 2025
Accepted: 15 May, 2025
Published: 30 Jun. 2025

the most important being Carl Linnaeus. Later[1], re-elevated taxonomy, as phylogenetic systematics, a central field of the biological sciences[2]. Taxonomic data are comprised of morphology, physiology, anatomy, behaviour, geography, phenology, molecular information, biological and ecological associations, imagery, and literature[3]. Taxonomists use these data to test species hypotheses for the classification of organisms. Taxonomists, thus, maintain a biological nomenclature and thereby provide an integrated biological vocabulary for communicating and describing biodiversity[4]. Taxonomy is particularly useful for understanding species on Red Data Lists and for identifying biodiversity hotspots and keystone species for prioritizing conservation efforts[5] as well as eventual establishment of protected areas, addressing cross-border concerns like the spread of alien invasive species[6] and the conservation of migratory species. Therefore, the taxonomic discipline is of immense importance for documentation, conservation, and sustainable use of biodiversity.

The family Zingiberaceae shows a wide variation of somatic chromosome number, ranging from 2n = 22 to 2n = 96. This variation in chromosome number is attributed primarily to intraspecific number variations. The base chromosome number of the family is reported to vary from x = 6 to x = 25[6].

Due to the importance of Zingiberaceae, many scientists and botanists have tried to estimate and classify members of the family. Discrepancies have been observed in the numbers of genera and species in Zingiberaceae[7] and in establishing taxonomic relationships among them. There is a continuous addition of new species and divisions among the Zingiberaceae. This vagueness has been attributed to the fact that Zingiberaceae are in the active stage of evolution[8] and relationships between several newly described genera and species are yet to be established[9]. The study of the family Zingiberaceae has proceeded slowly, and many genera are not yet studied[10]. The confusion and controversies over the phylogenetic relationship of species in Zingiberaceae necessitated the need for a comprehensive and integrated approach in the systematic resolution of the four members of the family. In recent times, the cytogenetic and phytochemical characters are very rare among the various parameters used by taxonomists for the establishment of interspecific relationships among flowering plants. Moreover, since the first classification of Zingiberaceae in the 1800s, the family has continued to be refined. There have been constant re-classifications of some species in the Zingiberaceae, especially using molecular analysis. In recent times, little or nothing has been reported on the four species of Zingiberaceae in South-Eastern Nigeria using cytological and phytochemical analysis about modern taxonomic practices. The objective of this study is to analyze the karyotype of four species of the Zingiberaceae family and evaluate their taxonomic significance in systematic botany.

MATERIALS AND METHODS

Study locations and duration: The study was carried out from January to August, 2024. The laboratory studies were carried out at the International Institute of Tropical Agriculture (IITA), Ibadan, Oyo State, Nigeria. Ibadan is located between Latitude 7°23°16"N and Longitude 3°53°47"E (NIMET, 2014). Herbarium preparations were carried out in the Laboratory of Plant Science and Biotechnology, Imo State University, Owerri.

Specimen collection: The herbarium specimens and living plants were collected from various parts of Imo State. More than 10 samples per species were collected for analysis. The preparation of herbarium specimens was followed using standard herbarium collection methods according to Islam *et al*.[11]. Aseptic polythene bags were used during the specimen collection to avoid damage to specimens during field collections. The flowers were preserved in specimen bottles with 70% alcohol concentration. Voucher specimens were prepared, authenticated, numbered, and deposited at IMSUH with voucher numbers 1025 to 1028 for reference.

Specimen identification: The identification of specimens was done by Prof. F.N. Mbagwu, a Plant taxonomist in the Department of Plant Science and Biotechnology, Imo State University, Owerri, and authenticated at the Forest Herbarium Institute, Ibadan, using identification keys and matching herbarium samples[2].

Chromosome preparation: For mitotic studies, actively growing root tips of plantlets developing from the rhizomes were harvested at different locations within Imo State. The root tips were cut to about 1-1.5 cm length and were pre-treated with saturated solutions of colchicine (8°C, 3 hrs) and fixed in 3:1 absolute ethanol: Glacial acetic acid (10°C for 16 hrs). After thoroughly washing three times in distilled water, the root-tips were re-suspended in freshly prepared Carnoy's fluid and stored at 12°C for 24 hrs. The root-tips were then fixed in 70% ethanol and were macerated in 20% HCL solution for 2 days. The root tips were stained in 2% propionic orcein-HCl mixture (9:1 v/v) (1-2 hrs at room temperature) and squashed in 45% propionic acid in clean, grease-free slides. Well-scattered metaphase plates (with properly condensed chromosomes) were observed under a microscope at a magnification of 1000× and photographed using a digital microscope (Celestron LCD Digital Microscope, Warsaw, Poland) Camera. Data for karyotype analysis of the investigated species were based on at least 20 independent plates from ten root tips, and data for B chromosomes were based on unbiased observations of over a hundred division stages from several root tips belonging to different plants under investigation[1].

Karyotype analysis: Chromosome counts were performed on 20 well-spread metaphase chromosomes from ten different root tips. For detailed karyotype analysis, values of measurements such as length of long arm (LA), length of short arm (SA), total length of chromosome (TL = LA+SA), standard deviation of chromosome length (SCL), mean chromosome length (XCL), centromeric index (CI = SA/TL×100), standard deviation of centromeric index (SCI), mean centromeric index (XCI), value of relative chromatin (VRC = ΣTL/n) were calculated. Karyotype asymmetry was evaluated using Huziwara's total form percent (TF (%)) and Zarco's intra and inter-chromosomal asymmetry indexes (A1 and A2)[4]. Classification of karyotypes according to their degree of asymmetry was calculated (Stebbins, 1971)[3]. The coefficients of variation of chromosome length (CVCL), chromosome index (CVCI), and asymmetric index (AI) were calculated based on Paszko (Paszko, 2006). Chromosomes were classified[3].

RESULTS AND DISCUSSION

Aframomum melegueta has 2n = chromosomes with one metacentric, two submetacentric and four subtelocentric chromosomes. Table 1. Length of long arm chromosomes (LA) ranged from 1.56-3.50 µm length of short arm chromosomes (SA) ranged from 0.71 to 1.31 µm (Table 1).

The total arm length of chromosomes (TL) ranged from 2.49 to 4.71 µm, Relative length [RL]ranged from 10.45 to 19.77%. Centromeric index (CI) ranged from 0.51 to 0.79 (Table 1).

Curcuma longa has a 2n = chromosome with three submetacentric and four subtelocentric chromosomes (2) and Table 2). The length of the long arm chromosome (LA) ranged from 2.5 to 4.48 µm. Lenght of short arm chromosome (SA) ranged from 0.71 to 2.25 µm (Table 2). The total arm length of chromosomes (TL) ranged from 3.22 to 6.73 µm. The relative length of chromosomes w 9.77 to 20.41% (Table 2). Centrometric index (CI) were 0.66-0.78 (Table 2).

For *E. cardamomum* chromosome number was 2n = with three metacentric, three submetacentric, and one subtelocentric chromosomes (Table 3). The length of the long arm chromosome (LA) was between 1.80 and 3.77 µm, and the length short arm (SA) was between 0.60 and 2.3 µm (Table 3). Total arm length (TL) was between 2.53 and 5.61 µm, and relative length (RL) was between 8.70 and 19.27%, while centrometric index (CI) was between 0.53 and 0.78 µm (Table 3).

Table 1: Karyotype analysis of *Aframomum melegueta*

Chromosome pair	SA±SD (µm)	LA±SD (µm)	TL±SD (µm)	RL (%)	CI	Chromosome type
1	0.81±0.10	2.53±0.05	3.34±0.09	14.02	0.76	Subtelocentric
2	0.93±0.07	1.56±0.05	2.49±0.10	10.45	0.63	Submetacentric
3	1.31±0.08	2.36±0.07	3.67±0,08	15.40	0.64	Submetacentric
4	1.21±0.08	3.50±0.08	4.71±0.09	19.77	0.74	Subtelocentric
5	0.78±0.10	2.91±0.07	3.69±0.09	15.48	0.79	Subtelocentric
6	0.71±0.07	1.80±0.05	3.51±0.10	1053	0.51	Metacentric
7	0.85±0.08	2.57±0.05	3.42±0.10	1435	0.75	Subtelocentric

Karyotype formula: 1m+2sm+4st, TL: Total arm chromosome, RL: Relative Length, CI: Centromeric index, SD: Standard deviation, SA: Length of short arm chromosome and LA: length of long arm of chromosome

Table 2: Karyotype analysis of *Curcuma longa*

Chromosome pair	SA+SD (µm)	LA±SD (µm)	TL±SD (µm)	RL (%)	CI	Chromosome type
1	0.91±0.08	3,31±0.05	4.22±0.60	12.80	0.78	Subtelocentric
2	0.71±0.07	2.51±0.05	3.22±0.50	9.77	0.78	Subtelocentric
3	1.01±0.10	3.10±0.07	4.11±0.70	12.47	0.75	Subtelocentric
4	0.8±0.10	2.71±0.05	3.51±0.80	10.65	0.77	Subtelocentric
5	1.53±0.08	3.45±0.07	4.98±0.91	15.10	0.69	Submetacentric
6	2.10±0.10	4.10±0.05	6.20±0.10	18.80	0.66	Submetacentric
7	2.25±0.10	4.48±0.06	6.73±0.10	20.41	0.67	Submetacentric

Karyotype formula: 3sm+4st, TL: Total arm chromosome, RL: Relative length, CI: Centromeric index, SD: Standard deviation, SA: Length of short arm chromosome and LA: length of the long arm of the chromosome

Table 3: Karyotype analysis of *Elettaria cardamomum*

Chromosome pair	SA±SD (µm)	LA±SD (µm)	TL±SD (µm)	RL (%)	CI	Chromosome type
1	1.80±0.100	3.77±0.81	5,57±0.10	19.13	0.68	Submetacentric
2	1.96±0.10	2.50±0.16	4.46±0.010	15.32	0.56	Metacentric
3	1.22±0.08	2.51±0.16	3.73±0.08	12.81	0.67	Submetacentric
4	2.31±0.08	3.30±0..18	5.61±0.04	19.27	0.59	Metacentric
5	2.1±0.10	2.91±0.07	4.51±0.05	15.50	0.53	Metacentric
6	0.80±0.07	1.80±0.05	2.53±0.04	8.70	0.68	Submetacentric
7	0.6±10	2.57±0.05	2.70±0.10	9.28	0.78	Subtelocentric

Karyotype formula: 3m+3sm+1st, TL: Total arm chromosome, RL: Relative length, CI: Centromeric index, SD: Standard deviation, SA: Length of short arm chromosome and LA: Length of long arm of chromosome

Table 4: Karyotype analysis of *Zingiber Officinale*

Chromosome pair	SA±SD (µm)	LA±SD (µm)	TL±SD (µm)	RL (%)	CI	Chromosome type
1	1.25±0.10	2.17±0.05	3.427±0.07	11.79	0.63	Submetacentric
2	1.10±0.19	3.31±0.07	4.41±0.08	15.21	0.75	Subtelocentric
3	2.70±0.08	3.10±0.08	5.80±0.05	20.00	0.53	Metacentric
4	2.30±0.08	3.75±0.07	6.05±0.05	20.86	0.62	Submetacentric
5	1.75±0.10	2.91±0.10	4.66±0.07	16.07	0.62	Submetacentric
6	0.61±0.10	1.81±0.05	2.42±0.08	8.34	0.75	Subtelocentric
7	0.51±0.08	1.73±0.06	2.24±0.05	7.72	0.77	Subtelocentric

Karyotype formula: 1m+3sm+3st, TL: Total arm chromosome, RL: Relative length, CI: Centromeric index, SD: Standard deviation, SA: Length of short arm chromosome and LA: Length of long arm of chromosome

In Z. *officinale*, chromosome numbers were 2n = with one metacentric, three submetacentric and three subtelocentric chromosomes (Table 4) and (length of long arm chromosome (LA) were between 1.73 and 3.75 µm and length of short arm (SA) were between 0.51 and 2.70 µm (Table 4). Total arm length chromosome (TL) ranged from 2.24 to 6.05 µm, and relative length (RL) was between 7.72 and 20.86%. Centromeric index (CI) was between 0.62 and 0.77.

The essence of this work is to reassess the four species of the family, Zingiberaceae, namely *Aframomum melegueta*, *Curcuma longa, Elettaria cardamomum*, and *Zingiber officinale,* with the aid of the cytological and phytochemical compositions in order to establish their taxonomic relationships for systematic consideration of the investigated taxa.

Based on the findings in this study, the karyotypic evidence of the four Zingiberaceae species showed that they possess submetacentric and subtelocentric chromosomes, indicating their ancestral relationship.

Similarly, the species *A. melegueta, E. cardamomum,* and *Z. officinale* possess metacentric chromosomes to affirm their taxonomic relationships. Based on their metacentric numbers obtained, *A. melegueta* and *Z. officinale* can be more closely related taxonomically than other species, and the absence of metacentric chromosomes observed in *C. longa* strengthens its taxonomic separation into a different genus.

Among the studied species, the chromosome number differs among the four species which was used to delineate them. The significant diversity recorded in the chromosome number of the studied Zingiberaceae is of taxonomic relevance. According to Jeon *et al.*[12] the diversity of chromosome number plays a significant role in separating plant species. Furthermore, Z. *officinale* 2n = 22, as reported in this study, has been reported in a previous study by Joseph *et al.*[13].

The reports of Islam *et al.*[10] showed the chromosome number of *Z. officinale* as 2n = 24. These differences could be as a result of the difference in the environment, as environmental factors have been proven to affect the chromosome number[5]. This study reveals that all the Zingiberaceae taxa studied are diploid species. The karyotype of *A. melegueta* is reported here for the first time. The total chromosome length (TL) indicated that the chromosomal length differs from each of the four species investigated, with *C. longa* having the largest chromosome, followed by *Z. officinale*. The chromosomes are smaller in *A. melegueta*. The variation in size of chromosomes can be used to delineate the species investigated. They were able to separate eight Euphorbia taxa they studied using their chromosome sizes. Similarly, Dutta and Bandyopadhyay[4] delineated members of *Veronieae* species they studied using their chromosome size. The variation in the chromosome arm has been reported to be due to the position of the centromere on its arm. The chromosome shape could help to know the relationship between species. The submetacentric and subtelocentric chromosomes are most common among the studied species. This showed that even in different species, the species are very similar in chromosomes at the family level. Metacentric chromosomes are most common among the studied species. This is in contrast with the statement of Jeon *et al.*[12] who stated that the metacentric shape of chromosomes is common in plants.

The somatic chromosome number of the four species of Zingiberaceae differs from each other. In *Z. officinale,* the 2n = 22 as reported is consistent with other findings reported by some authors[3]. These differences from 2n = 24 reported by Jeon *et al.*[12]. Similarly, the somatic chromosome number of 2n = 63 recorded in *C. longa* has also been reported by other researchers, such as Chen and Xia (2010). The karyotype analysis of *A. melegueta* was first reported here. The types of chromosomes observed were mostly subtelocentric followed by submetacentric; only one metacentric chromosome was recorded submetacentric, subtelocentric. The chromosome size variation from other authors, such as Islam *et al.*[10]. In *E. cardamomum*, different authors have reported different chromosome numbers in the past. Joseph *et al.*[13] have reported chromosome number of 2n = 48,52. At this point, there is a difference between the findings of this study *E. cardamomum* (2n), and the other authors. This calls for further cytological research on the species. The type of chromosomes observed in *E. Cardamomum* are metacentric, submetacentric, and subtelocentric, with subtelocentric occurring once.

CONCLUSION

Taxonomically, some phytochemicals identified among the four species of Zingiberaceae studied revealed that they belong to a common ancestor. The presence of oleic acid in *C. longa*, *E. cardamomum,* and *Z. officinale* indicates a closer relationship than. *E. cardamom*. Based on this, the species investigated showed a taxonomic relationship with each other using their phytochemical position. Based on the

findings of this study, it is suggested that cytogenetic and GC-MS analyses be conducted on other plant species not included in this research to further explore and validate the findings. Additionally, it is recommended that the molecular characteristics of the plant species utilized in this study be examined, as this would provide a more robust foundation for reinforcing the taxonomic classification and understanding of these species.

SIGNIFICANCE STATEMENT

This study discovered the cytogenetic and chemical diversity within selected species of the Zingiberaceae family that can be beneficial for resolving longstanding taxonomic ambiguities and enhancing classification systems in Systematic Botany. Through karyotype analysis and GC-MS profiling, the study provides robust evidence supporting the genetic and chemical basis for differentiating closely related taxa. These findings offer a deeper understanding of interspecies relationships and contribute to clarifying evolutionary lineages within the family. Furthermore, the combined use of cytogenetic and metabolomic tools presents a novel integrative approach for botanical classification. This study will help researchers to uncover the critical areas of species divergence and phylogenetic placement that many researchers were not able to explore. Thus, a new theory on Zingiberaceae taxonomy may be arrived at.

REFERENCES

1. de Storme, N. and A. Mason, 2014. Plant speciation through chromosome instability and ploidy change: Cellular mechanisms, molecular factors and evolutionary relevance. Curr. Plant Biol., 1: 10-33.
2. Doyle, J.J., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull., 19: 11-15.
3. Dũng, N.X., P.X. Truong, P.T. Ky and P.A. Leclercq, 1997. Volatile constituents of the leaf, stem, rhizome, roof and flower oils of *Curcuma harmandii* Gagnep. from Vietnam. J. Essent. Oil Res., 9: 677-681.
4. Dutta, M. and M. Bandyopadhyay, 2014. Comparative karyomorphological studies of three edible locally important species of *Allium* from India. Nucleus, 57: 25-31.
5. Edwards, J.D. and S.R. McCouch, 2007. Molecular Markers for use in Plant Molecular Breeding and Germplasm Evaluation. In: Marker-Assisted Selection. Current Status and Future Perspectives in Crops, Livestock, Forestry and Fish, Guimarges, E., J. Ruane, B.D Schert, A. Sonino and J.D. Dargie (Eds.), FAO, Rome, Italy, ISBN: 978-92-5-105717-9, pp: 29-49.
6. Gershenzon, J. and N. Dudareva, 2007. The function of terpene natural products in the natural world. Nat. Chem. Biol., 3: 408-414.
7. Gilani, A.H., Q. Jabeen, A.U. Khan and A.J. Shah, 2008. Gut modulatory, blood pressure lowering, diuretic and sedative activities of cardamom. J. Ethnopharmacol., 115: 463-472.
8. Huziwara, Y., 1962. Karyotype analysis in some genera of compositae. VIII. further studies on the chromosomes of aster. Am. J. Bot., 49: 116-119.
9. Ilic, N., B.M. Schmidt, A. Poulev and I. Raskin, 2010. Toxicological evaluation of Grains of Paradise (*Aframomum melegueta*) [Roscoe] K. Schum. J. Ethnopharmacol., 127: 352-356.
10. Islam, M.A., K. Kloppstech and E. Esch, 2005. Population genetic diversity of *Curcuma zedoaria* (Christm.) Roscoe. A conservation prioritized medicinal plant in Bangladesh. Conserv. Genet., 6: 1027-1033.
11. Islam, M.A., A. Meister, V. Schubert, K. Kloppstech and E. Esch, 2007. Genetic diversity and cytogenetic analyses in *Curcuma zedoaria* (Christm.) Roscoe from Bangladesh. Genet. Resour. Crop Evol., 54: 149-156.
12. Jeon, W.Y., M.Y. Lee, I.S. Shin, S.E. Jin and H. Ha, 2015. *Curcuma aromatica* water extract attenuates ethanol-induced gastritis via enhancement of antioxidant status. Evidence-Based Complementary Altern. Med., Vol. 2015. 10.1155/2015/582496.
13. Joseph, R., T. Joseph and J. Jose, 1999. Karyomorphological studies in the genus *Curcuma* Linn. Cytologia, 64: 313-317.