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Karyotype analysis of *A. indica* and *B. nitida* and their taxonomic relationship

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Abstract: *Chromosome characters of two indigenous medicinal plants of high repute were studied. Variation in chromosome features is believed to have accompanied evolutionary divergence of many plant and animal species. This aspect of the research investigated the karyomorphological details of two species of medicinal plants (A. indica and B. nitida) from different families and their possible taxonomic relations using actively dividing root meristems and a high-resolution image-based cytogenetic system. The conventional squashing in aceto-orcein of root tissues after heating was used. The tissues were photographed under Nikon Universal Microscope, equipped with an MC 100 camera. The chromosomes were then measured under x400 magnification using a micrometer. The results revealed that the mitotic phases varied with species. Similarly, the arm ratio, r-value, centromeric index, coefficient of variation, total form, intra-chromosomal index and inter-chromosomal index varied with the accessions. All examined cells displayed a varied chromosome number and karyotype formula. The highest long arm chromosome was recorded in B. nitida with a mean value of 37.25± 0.88 µm while while A. indica registered the minimum (9.64±0.33 µm). The mean Arm Ratio of the chromosomes was highest in A. indica (2.048± 0.12 µm) while the least was recorded from B. nitida (1.450± 0.13 µm). A parallel pattern was observed for the Total length dimension, in which B. nitida attained the highest value (62.95 ± 1.43 µm) and A. indica the lowest (29.39 ± 1.57 µm). From the perspective of the centromeric index, the B. nitida population manifested the highest magnitude of this parameter (26.389± 0.05 µm), whereas A. indica presented the lowest value (10.128± 0.08 µm). These differences could be explored for the improvement of the medicinal plants cultivated in Nigeria.*

Keywords: Karyotype analysis, *A. indica*, *B. nitida*, taxonomic relationship

INTRODUCTION

Plant and its materials have been used by human for various purposes such as construction, food, medication, clothing and so on. Most of these plants are cultivated for their therapeutic properties for ages. Sometimes, two or more plants can have the ability to cure a particular disease or diseases [1]. Also, combination of two or more plants tends to cure a disease. Researchers have attributed the ability of different plants species to cure same diseases to their ancestral connection, yet there is little or no information on how these plants taxonomically relate[2]. The lack of detailed understanding of the cytogenetic analysis and phytochemical constitution of these plant species makes it impossible to properly identify them and also establish their relationship. The cytogenetic analysis of some plant species showed variation in chromosome number and morphology which has been attributed to the production of certain bioactive compounds and hence, contribute to their medicinal properties. Therefore, understanding the chromosome number, morphology and karyotype characteristics of the investigated plant species will help understand their medicinal potentials and establish any relationship among them[4].

A. indica, also known as Indian Lilac is a tree in the mahogany family Meliaceae [5] It is commonly called as Neem, Nim tree, and It is one of two species in the genus *Azadirachta*. In Nigeria, it is also known as Wonder leaves. Although, it is mostly found in the Northern region where it grows tall and produces fruit It is called by the native name Dogoyaro, it is typically grown in tropical and semi-tropical regions, is a herb known to have numerous health benefits [6] Neem is a fast-growing tree that can reach a height of 15–20 metres (49–66 ft), and rarely 35–40 metres (115–131 ft). It is evergreen, but in severe drought it may shed most or nearly all of its leaves. The neem tree is very similar in appearance to its relative, the Chinaberry (*Melia azedarach*) [7].

Baphia nitida Lodd**.** belongs to the family Leguminosae-Papilionoidea. A shrub or tree to 10m high with trunk to 45cm diameter with slender branches forming an umbrella-shaped crown; usually an under-story tree of wetter parts of the coastal area and into the interior of the rain forest zones. It is an erect small tree native to central-West Africa. It grows up to 10m tall and 45cm across and is usually planted as ornamental shade tree or hedge[8]. The bark and heartwood are great source of a high-quality red dye used to dye raffia and cotton textiles. Camwood is also used as a medicinal plant. In particular, it has been used in traditional African medicine. The leaves have inflammatory activities, antidiarrheal effects and analgestic activities. Powdered heartwood can be made into ointment with shea butter for sprains, swollen joints and rheumatic pains. Roots have medicinal properties as well. The twigs are used as chewing sticks. The seeds are edible. Other names are, Barwood, Dolo, Doro, African Sandalwood. Therefore, this study is poised to understanding the taxonomic relationships existing among the selected medicinal plants using their cytological characters compositions with the aid of modern technology.

MATERIALS AND METHODS

Plant Collection

All the plant samples were collected from various locations in Imo State. *Baphia nitida* was collected from Mr.Chisom Odom Farm in Obizi Ezinihitte Mbaise LGA. *Azadriactha indica* was

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obtained from Imo State University Staff Quarters. The Botanical Name, Common Name, Location and Coordinates of the four species is shown in Table 1.

Table 1. Location of the four medicinal plant species and their coordinates

Botanical Name	Common Name	Location	Coordinates
Azadriactha indica	Neem	Imo State University Demonstration Garden	$5^o 30^I 27.8183^{II}$ N Lat $7^o 02^1 25.5487^{11}$ E Log
Baphia nitida	Camwood	Obizi, Ezinihitte Mbaise LGA	$5^o 25^I 24^{II}$ N Lat $7^o 43^1 29^{11}$ E Log

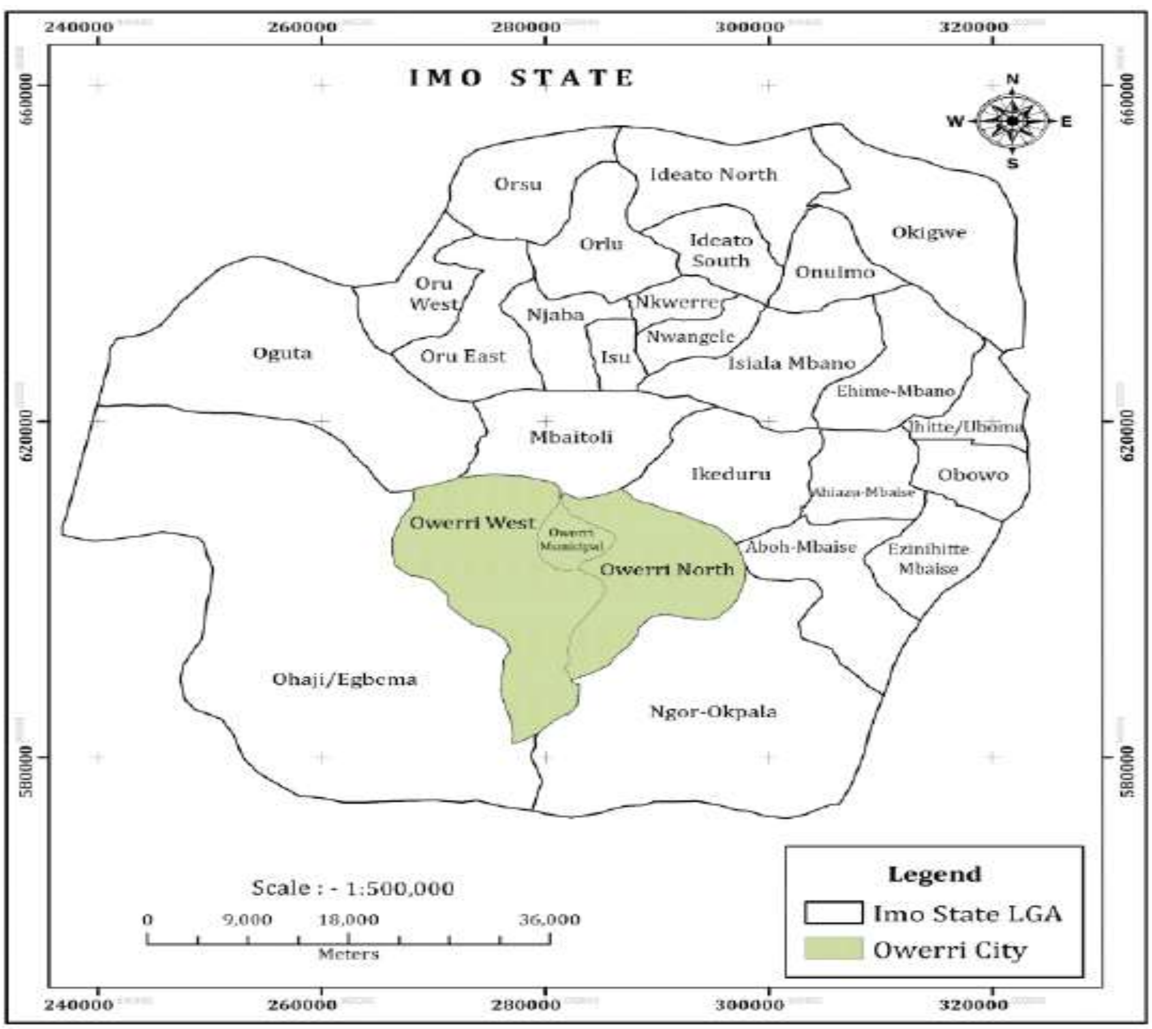

Figure 1: Map of Imo State showing collection areas (Source: Onwuadiochi, et al., 2021)

Preliminary Study Site
For easier accessibility of the young actively growing root tips, the stems of the plant were planted in perforated bags behind the Department of Genetics and Biotechnology, University of Calabar, since the plant requires a shaded environment to thrive well. The seeds were also sprouted in a water-soaked cotton wool placed on petri dishes and kept in a dark cupboard in the laboratory for easy sprouting.

Materials Used
Root tips of *Azadiractha indica* and *Baphia nitida, Distilled* water, Water bath, Petri dishes, Test tubes and beakers, Slides and cover slips, Specimen/reagent bottles, Forceps, Surgical blade, compound Microscopes, Filter paper, Nail vanish, Orcein stain, 8-hydroxyquinoline, Hemoxyline, Colchicine, 70 and 100% ethanol, Polythene bags, Cotton wool, Masking tape and biro.

Identification and Authentication of the two Selected Medicinal Plants investigated
Specimen identification and confirmation was done by using Flora of West Tropical Africa and were authenticated by Professor F. N. Mbagwu of the Plant Science and Biotechnology Department in Imo State University and voucher specimens of the two investigated plants were prepared with voucher numbers IMSUH-436 and IMSUH-464 for *A. indica*, and *B. nitida*, which were deposited for future reference at the Imo State University Herbarium. The leaves, stem and roots were shade dried at room temperature and powdered with mortar and pestle kept in an amber colour container [8].

Plate 1: Morphology of A =*Azadriactha indica* and B= *Baphia nitida*

Cytological characterization of the species investigated

METHODS

Harvesting

Young healthy root (about 15mm) were carefully collected at two-hour interval from 7:00 and 9:00am each day from the roots of *Azadiractha indica,* and *Baphia nitida.*

Pretreatment

The harvested root tips were rinsed twice in distilled water and pre-treated in 0.002ml solution of 8-hydroxylquinoline (0.058g dissolved in 200ml of distilled water for four (4) hours.

Fixation

The pre-treated root tips were rinsed twice in distilled water and fixed in 1:3 glacial acetic alcohols (1-part glacial acetic, to 3 parts ethanol). The fixative was freshly prepared for use and the root tips were fixed at room temperature for 24 hours, after which they were stored in 70% ethanol for further use.

Hydrolysis

The fixed root tips were again rinsed twice in distilled water then hydrolyzed in water bath, and controlled at 60°C for six (6) minutes.

Storage

The root tips were stored in 70% ethanol solution, and preserved in a refrigerator until it was needed for squashing.

Squashing and Staining

The hydrolyzed root tips were rinsed twice in distilled water, placed on a clean grease free slides (one root tip per slide) and excess fluid was removed using surgical blade. The apical 1mm (whiter and denser) portion of the root tips were carefully cut off on the slide. One to two drops of 1% aceto orcein was added to the specimen and the material was macerated thoroughly. A thin cover slip was laid on top of the specimen and the slide was placed in a folder filter paper, and thumb pressure was applied to remove excess stain.

The cover slip was tapped gently with the blunt end of a biro. Tapping continued until the materials became well spread out and hardly visible. The corner slip was sealed with nail vanish and then viewed under the microscope.

Viewing of the Slides
The slides were placed on the stage of the microscope and then adjusted for proper and clearer views at various magnifications; Photomicrographs of the cells at various stages of mitosis were taken at x100 with oil immersion.

Karyotype Analysis
Fresh root tips of 1 – 1.5 cm long was cut from rapidly growing seedlings (2 – 3 weeks after planting). The root tips was pretreated with 0.5 g of 8 – hydroxyquionolene in 100 ml of distilled water at room temperature for 3 hours following the method [4]. Five well-spread metaphase plates were analyzed in terms of

1. Short arm length (S)
2. Long arm length (L)
3. Total length of chromosome (TL)
4. Relative value (RV)
5. Arm ratio (AR)
6. Centrometric index (CI)
7. Total form (TF)
8. Karyotype formula

The best metaphase plates in terms of clarity was photographed using an Amscope digital Camera 1000MA coupled to the microscope and laptop and scanned at 1000 – resolutions.The conventional squashing protocol in aceto –orcein of the root tissues after heating was adopted. The tissues were photographed under Amscope Microscope equipped with 1000MA camera. The chromosomes (shown in the plates 1 – 4) were measured using a IScapture image program software application tools. All the forest species study were diploid with somatic chromosome number for both seed sprouted and stem sprouted root tips.

All measurements were recorded using the software images. Chromosomal morphology was described using nomenclatures proposed by Levan et al, 2004 while numerical characterization was done using the clipman tool.

Statistical Analysis of Data
The data generated from the study on short arm length, long arm length, arm ratio, R-value and centrometric index and karyotyping was analyzed and generated using ISCapture image software program. PAST 4 was employed for coordinate locations of chromosomes.

RESULTS AND DISCUSSION

The results of the karyotype details of the crude extract of *A. indica* is shown in Table 1. The analysis of variance performed on the karyotypic parameters of the examined species revealed highly significant interspecies differentiation at the $p < 0.01$ threshold for total chromosomal length (TL), long-arm length (LA), short-arm length (SA), Relative Length and centromeric index (CI). The result indicated that the species has a chromosome count of $2n = 2x = 24$, having a karyotype formula of 10m + 2 sm.

The total mean length of short arm chromosome in the species ranged from 0.18±0.07 µm to 2.11±0.07 µm revealing a metacentric chromosome morphology. The length of long arm chromosome in *A. indica* also ranged from 0.90±0.07 µm to 5.91±0.07 µm in size. The total arm chromosome ranged from 0.35±0.07 µm to 4.01±0.09 µm revealing a metacentric chromosome morphology in the species.

The longest chromosome constituted 19.45% of the genome, while the shortest occupied 0.35 % with a Metacentric chromosome morphology. With respect to the Centrometric index, the results showed that the highest CI was 0.84 while the least was 0.32 respectively. The cytogenetic details obtained from this species shows that *A. indica* was mostly a metacentric karyotype morphology.

Table 1: karyotype details of the crude extract of *A. indica*

Chromosome pair	SA±SD (µm)	LA±SD (µm)	TL±SD(µm)	RL (%)	CI	Chromosome morphology
1	0.51±0.30	1.33±0.05	3.34±0.09	11.02	0.66	Metacentric
2	0.41±0.05	2.22±0.05	2.49±0.10	9.45	0.53	Metacentric
3	0.31±0.03	5.36±0.07	3.67±0,08	12.30	0.74	Metacentric
4	0.01±0.05	4.50±0.08	4.71±0.09	15.77	0.84	Submetacentric
5	0.58±0.07	5.91±0.07	3.69±0.09	13.48	0.59	Metacentric
6	0.51±0.05	2.80±0.05	3.51±0.10	12.53	0.41	Metacentric
7	0.71±0.30	1.53±0.05	3.64±0.09	10.02	0.68	Metacentric
8	0.31±0.05	2.02±0.05	2.29±0.10	19.45	0.43	Metacentric
9	0.51±0.03	2.46±0.07	3.77±0,08	11.30	0.44	Metacentric
10	1.06±0.05	3.10±0.08	4.01±0.09	11.77	0.34	Submetacentric
11	2.11±0.07	3.39±0.09	0.46±0.07	0.35	0.72	Metacentric
12	0.18±0.07	0.90±0.07	0.35±0.07	0.42	0.32	Metacentric

Karyotype analysis of *A. indica; Karyotype formula = 10 m + 2 sm*
TL= Total arm chromosome, RL= Relative Length, CI= Centrometric index
SD= Standard deviation, SA=Length of short arm chromosome, LA=length of long arm chromosome

Plate 2*: Azadiractha indica* chromosomes

The results of the karyotype analysis of the crude extract of *B. nitida* is shown in Table 2. The results revealed a significant variation in the twenty two (22) Chromosome pairs and the assayed parameters. The result indicated that the species has a chromosome count of 2n=2x=44, having a karyotype formula of 13m + 9 sm.

The length of short arm chromosome in the species ranged from 0.12±0.09 µm to 3.72±0.09 µm indicating a metacentric chromosome morphology. The length of long arm chromosome in *B. nitida* also ranged from 0.10±0.05 µm to 4.51±0.04 µm. The Total arm chromosome ranged from 2.32±0.09 µm to 7.11±0.06 µm revealing a Metacentric chromosome morphology in the species. Similarly, the relative length of the *B. nitida* revealed a range of 11.77 % to 19.80 % with a Metacentric chromosome morphology. With respect to the Centrometric index, the results showed that the highest CI was 0.98 while the least was 0.23 respectively.

Table 2: Karyotype analysis of the crude extract of *B. nitida*

Chromosome pair	SA+SD (µm)	LA±SD (µm)	TL±SD (µm)	RL(%)	CI	Chromosome morphology
1	0.81±0.06	2.21±0.04	6.22±0.06	14.80	0.88	Metacentric
2	0.61±0.05	2.71±0.05	3.32±0.05	11.77	0.68	Metacentric
3	0.31±0.11	3.40±0.06	5.11±0.07	15.47	0.55	Metacentric
4	0.70±0.05	2.81±0.04	7.11±0.06	13.65	0.87	Metacentric
5	1.43±0.06	3.45±0.06	5.58±0.06	17.10	0.63	Submetacentric
6	2.12±0.09	3.00±0.05	7.10±0.09	16.80	0.73	Submetacentric
7	0.81±0.06	4.51±0.04	6.22±0.06	17.80	0.91	Metacentric
8	0.61±0.05	2.71±0.05	3.32±0.05	10.77	0.58	Metacentric
9	0.31±0.11	3.30±0.06	5.11±0.07	13.47	0.65	Metacentric
10	0.70±0.05	2.61±0.04	7.11±0.06	12.65	0.47	Metacentric
11	1.43±0.06	3.31±0.06	5.58±0.06	18.10	0.73	Submetacentric
12	0.12±0.09	3.12±0.05	7.10±0.09	19.80	0.43	Submetacentric
13	2.02±0.09	3.03±0.05	7.10±0.09	13.80	0.83	Submetacentric
14	0.51±0.06	2.01±0.04	3.22±0.06	13.80	0.98	Metacentric
15	0.41±0.05	2.51±0.05	3.02±0.05	11.77	0.38	Metacentric
16	0.21±0.11	2.20±0.06	4.11±0.07	14.47	0.95	Metacentric
17	0.80±0.05	2.61±0.04	5.01±0.06	14.61	0.27	Metacentric
18	1.33±0.06	2.05±0.06	3.48±0.06	18.10	0.23	Submetacentric
19	0.52±0.09	0.20±0.05	5.10±0.09	15.80	0.93	Submetacentric
20	3.72±0.09	0.10±0.05	7.00±0.09	11.81	0.43	Submetacentric
21	0.12±0.09	0.20±0.05	7.20±0.09	19.09	0.98	Submetacentric
22	0.82±0.09	0.42±0.09	2.32±0.09	12.08	0.76	Metacentric

Karyotype formula = 13m + 9 sm

TL= Total arm chromosome, RL= Relative Length, CI= Centrometric index, SD= Standard deviation
SA=Length of short arm chromosome, LA=length of long arm chromosome

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Plate 3*: Baphia nitida chromosomes*

DISCUSSION

Cytogenetic and karyomorphological investigations have long been regarded as indispensable tools in elucidating phylogenetic affinities, genomic divergence, and evolutionary trajectories within and among plant taxa. Systematic scientists posit that chromosomal attribute (when interpreted alongside genetic and morphological criteria) serve as highly reliable indicators for assessing kinship relationships among congeneric species, given that the structural configuration of chromosomes often mirrors their evolutionary proximity.

Somatic chromosome count, base number and ploidy level of species form the primary basis of cytotaxonomy. Identifying chromosomal variations is crucial for the evolutionary analysis, research, and breeding of new cultivars [8]. Karyotypic parameters are one of the crucial indicators for classifying and identifying plants, thus enabling the study of their genetic diversity in cytological terms [2]. This aspect of the research was undertaken to ascertain the karyomorphological details of the two species of medicinal plants from different families and their possible taxonomic relations.

As chromosomes represent the fundamental carriers of hereditary information, any fluctuation in their architecture (including number, form, and proportional dimensions) inevitably reflects underlying genomic asymmetries, thereby manifesting as phenotypic disparities across populations and species.

The present study showed that the chromosomes of the two medicinal plants studied were mainly of the m and sm types, and no satellite chromosomes were found. Furthermore, the symmetry of karyotypes was generally neat. The number of karyotypes within a species is m-type > sm-type, and the number of m chromosomes is greater than that of the sm chromosomes. This conforms with the report of [9].

The chromosomes numbers in all the plant species varied significantly. The variations in chromosome numbers reflected frequent dysploidy which is an increase or decrease in the chromosome number as a result of structural rearrangements. Similarly, the differences in chromosome number and chromosome morphology found among the species in this study indicate that chromosome structural changes may be used to distinguish species that are very similar to each other and cannot be separated using morphological characters. This agrees with the report of [10] in their study on karyotype analysis of three species of *Allium* (*Amaryllidaceae*) from Thailand.

Contrary to this findings, [11] found similar chromosomal counts in their study on comparative Chromosome Morphology and Karyotype Study in Two Varieties of *Allium cepa*. The difference in chromosome numbers between the species observed in this study indicated intraspecific variation at ploidy level and basic chromosome number among the species. This report corroborates the findings of [12] in *O. basilicum* var. *crispum*, but in discord with the report of [13] from North India.

The study established that different karyotype formulae were observed for each medicinal plant species studied. The karyotype formula of *A. indica* and *B. nitida* did not increase exponentially. This may be because of the chromosome type changed after doubling because of the effect of colchicine treatment, or the sm-type chromosome was not doubled entirely, whereas the m-type chromosome was doubled greatly, resulting in the karyotype formula not increasing exponentially. Similar opinion had been reported by [14]

This is in agreement with the report of previous findings of [4] who reported variation in chromosomes formulae in the genus *Ocimum.* According to Zhang *et al.* (2026), karyotypic and genome size differences indicates existence of cryptic genetic variations among the cultivars. As argued by previous authors, variation in chromosome can also be attributed to environmental conditions of the species under study [15]. [16] in their study on impact of temperature on karyotype differences in *Cynodon dactylon* of different ploidy levels at different latitudes also reported variation in chromosome resulting from temperature which conforms with the findings in this study.

[3] performed a karyotype analysis of existing *Chara* species of different ploidies and reported different karyotype formula ratios and karyotype types which supports the findings in this study. The karyotype analysis of *Hemerocallis* of different ploidy levels was performed by [5] and although different materials of the same ploidy level showed considerable differences, the proportion of karyotype formulas and karyotype types differed across different ploidy levels which aligns with the present study.

Therefore, heterozygosity and polymorphism exist in polyploids that have evolved over a long period in nature [8] thus leading to great variations in their karyotype formulas and karyotype categories. The variation in karyotype of different species could be brought about by dysploidy, pericentric inversion, deletion, unequal translocation, spontaneous mutation, interspecific hybridization etc

Such discrepancies in karyotype formula were probably due to differences in analyzed materials and mitotic stages used, and difficulty in identifying chromosomes using the classical staining technique before [1]. This observed difference could mainly be related to variation in the chromosome condensation levels of measured cells [12]

Mean length of short and long arm chromosome observed in this study indicated that polyploid species are often of smaller chromosome size than the diploid, this result agrees with [3]. The total chromosome length observed in the species and their hybrids in this study is in agreement with the report of [11] on total chromosome length recorded in *Ocimum* species studied, which was between 39.00 µm to 70.80 µm.

High mean centromeric index (CI) recorded in these two species could signify the primitiveness of these studied species and common ancestor. This aligns with the report of [1] in their study on Cross-species chromosome painting among 16 representative species of *Ipomoeeae*. Similarly, [14] reported that high centromeric index value denotes high symmetric karyotype, which is a primitive condition among the species. This result is in accordance with the reports from previous researchers [16]

A. indica species are characteristically diploid with 2n = 24 chromosomes, a feature consistently documented across a broad array of studies. Findings from the present investigation reaffirm this genomic stability in this species. The karyotypic assessment of the two studied species (*A. indica*, *B. nitida*) revealed a remarkable degree of morphological similarity, echoing the structural congruence previously observed in related investigations [17].

Chromosomes of *A. indica*, and *B. nitida* were characteristically small, and in the present study their lengths ranged from 0.90 µm to 2.12 µm a pattern highly consistent with the values reported by [15] who recorded chromosome lengths between 0.97 and 2.75 µm in their study on impact of temperature on karyotype differences in *Cynodon dactylon*.

The comparative karyotype analyses further demonstrated that the species possessed highly symmetrical complements, with most of the chromosomes classified as metacentric; neither telocentric nor acrocentric chromosomes were detected. This structural harmony aligns closely with the findings of [11] who likewise reported predominantly symmetric and morphologically similar karyotypes across multiple populations.

The disparity index (DI) has been reported as a useful tool to differentiate quantitatively and closely related karyotypes belonging to the same clan of symmetry [3] The high values of DI observed in this study suggests high levels of karyotype differentiation.

The intrachromosomal index (A1) and interchromosomal index (A2) showed slight variations between the medicinal plant species studied. Variations in long and short arm lengths within and between populations form the basis of morphological variations among different accessions of the species observed in this study. These variations could be used to determine the slight differences among populations [9].

Against this comparative backdrop, the present study demonstrates that the species under investigation exhibits an even more symmetrical karyotype than any previously documented species [12]. All chromosomes across all examined species were mostly metacentric, implying an extraordinarily stable and primitive cytogenetic architecture. Collectively, the results of this study highlight the critical role of cytogenetic approaches in transcending purely descriptive taxonomy by providing deeper insights into evolutionary processes, population structuring, and genome-level adaptations.

Generally, crop improvement techniques such as selection and hybridisation depend, to a large extent, on the existence of variability. Genetic diversity or variations that relate to karyotype forms have been employed as a useful tool in assessing the crop improvement potentials of some crops [11].

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