Karyo-Morphology and Nucleoli Analysis of Commelina L. (Commelinaceae) from Ethiopia

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Abstract

Background

With about 100 species, *Commelina* is the largest genus of Commelinaceae in Africa. Although medicinal and economic benefits had been studied extensively, little is known about its cytological analysis. Hence, this study will focus on chromosome and nucleoli analysis of selected species of Commelina L. Somatic chromosomes were prepared from root tips that emerged from the nodes of stem cuttings that were made to stand submerged in water. The roots were pretreated in 8-hydroxyquinelin 3-5 hrs followed by fixation in 3:1 ethanol: acetic acid for 1-24 hrs at 4^{O}C. Air-dry slides were prepared following cellulase and pectinase maceration at 37^{O}C, the preparation was stained in Giemsa stain (PH 6.4), rinsed and mounted. Nucleoli were stained in silver nitrate solution.

Results

Chromosome numbers and Karyotype formula of the four species were found as *C. africana* 2*n*=2*x*=30 (12m + 10sm + 8st), *C. benghalensis* 2*n*=6*x*=66 (36m + 24sm + 6st), *C. diffusa* (Ginchi) 2*n*=66 (28m +26sm + 12st), *C. diffusa (*Jimma*)* 2*n*=2*x*=30 (10m + 8sm + 12st) and *C. subulata* 2*n*=2*x*=30 (18m +10sm + 2st). According to Stebbins karyotype asymmetry, the karyotypes of *C. africana* and *C. subulata* were 2A type, while that of *C. benghalensis* and *C. diffusa* (Ginchi) were 2B type. 3A asymmetry type was obtained for *C. diffusa* (Jimma). Karotypes of *Tradescantia* were found to be monomodal for the *Commelina* species of the studied plant materials. Satellites were observed for species *C. africana* and *C. diffusa* with variation in number ranging from 2 to 6. The maximum number of nucleoli observed varies from two to four for *Commelina*.

Conclusions

This study reported karyotype and nucleoli of the Ethiopian Commelinaceae for the first time. The current investigation can be considered as an additional karyotype data to the earlier meiosis report for Ethiopian materials.

Background

The family Commelinaceae, commonly known as the spiderwort, is known to possess 41 genera and 650 species throughout the world [1], with main distribution in tropics and subtropics that extends into northern temperate regions. The family is represented by 9 genera and 56 species in Ethiopia [2].With about 100 species, *Commelina* is the largest genus of Commelinaceae in Africa. At least 65 species occur in the combined areas of the Flora of Tropical East Africa (Kenya, Uganda, and Tanzania) and Flora Zambesiaca (Malawi, Mozambique, Zambia, Zimbabwe, and Botswana) [3, 4]. About 19 species have been identified in Ethiopia and Eritrea including 2 un-described species that are endemic to Ethiopia [5].

The genus *Commelina* has several medicinal significances. *C. benghalensis* is used to treat bed sores, breast sores and pimples in Pakistan [6]. In East Africa, the sap of *C. benghalensis* leaves and stems is used to treat ophthalmia, infertility in women, leprosy, sore throat and burns, and the liquid contained in the flowering spathe is used to treat eye complaints in Zanzibar [7]. People from Nepal use a paste derived from the plant to treat burns, and indigestion with a juice produced from the roots [8]. In China, *C. diffusa* is used as a medicinal herb with febrifugal and diuretic effects [9].

C. benghalensis is known for its medicinal purposes as they have already been reported in treating hypertension, burns, leprosy, sore throats, cataplasm, and wound healing [10]. Numerous compounds have been identified from the vegetative and flower parts of *Commelina benghalensis* including noctacosanol, n-triocotanol, stigma-sterol, compesterol and hydrocyanic acid [11]. Phyto-chemical screening also revealed the presence of many secondary metabolites like phlobatannins, carbohydrates, tannins, glycosides, volatile oils, resins, balsams, flavonoids and saponins [12]. Presence of flavonoids, for example, indicates the plant might have an antioxidant, anti-allergic, anti-inflammatory, anti- microbial or anti- cancer activity [13]. In addition, *C. benghalensis* has analgestic action that proves the folkloric use in pain management [14].

Although several medicinal and economic uses of the family Commelinaceae are known, little has been done in cytological analysis in Ethiopia. Previous research done only focused on chromosome number of four species included in this study based on meiosis and voucher specimen were taken only from the Harar Province except for *Cyanotis barbata* D. Don which was from Debrezeit [15]. The present study was focused on chromosome number, karyotype analysis and silver staining. Here the chromosome number, ploidy level, karyotype and number of nucleoli organizing region (NORs) of these species of Commelinaceae are described. Such study can help to expand the current cytological knowledge of the Ethiopian Commelinaceae with further contribution for phylogenetic and biosystematics research.

Results

The *Commelina* species contain metacentric, sub-metacentric and sub-telocentric chromosomes with variation in number and morphology (Table 1).

C. africana

Somatic chromosomes observed from more than five intact cells showed that *C. africana* collected from Addis Ababa contained a diploid number of 2*n*=2*x*= 30. Because of its better chromosome spread and clear chromosome morphology a cell with 29 chromosome number is shown in Figure 1A and a karyotype constructed from same cell in Figure 1B. This variant of the species consisted of six pairs of metacentric chromosomes (pairs 1 to 6), five pairs of sub-metacentic chromosomes (pair numbers 7 to 11) and four sub-telocentrics (pairs 12 to 15) (Figure 1B). The karyotype formula is, therefore, 12m + 10sm + 8 st (Table 1). According to Stebbins karyotype classification, this species belongs to 2A type with asymmetry index of 65.19 (Table 1). Small satellites were also

observed at the tip of the short arm of a pair of chromosomes (Figure 2A) and these correspond to the 8th pair on the karyotype (Figure 1B). Figure 2B shows three interphase nucleoli, from which it can be assumed that the maximum number of nucleoli and so chromosome satellites for this species is four.

Length wise measurement of metaphase chromosomes revealed a value range from the smallest 4.53µm to largest 7.5µm (Table 1). Total length of whole chromosome complement (2*n*) was 176.72µm. The chromosomes were medium in size with an average length and asymmetry of 5.89µm and 1.87, respectively.

C. benghalensis

According to somatic chromosome analysis of the root tips, this species possesses 2*n*=6*x*=66 (Figure 1C and Table 1). The karyotpe constructed based on calculated arm ratio and chromosome size revealed the presence of eighteen metacentric chromosome pairs (pair numbers 1, 3, 4, 5, 8, 11, 14, 15, 16, 18, 22, 24, 25, 29, 30, 31, 32, 33), twelve sub-metacentrics (pair numbers 6, 7, 9, 10, 12, 17, 19, 20, 21, 23, 27, 28) and 3 sub-telocentric pairs (pairs 2, 13, 26) (Figure 1D). The karyotype formula was, therefore, 36m + 24sm + 6st. As to Stebbins method of classification, *C. benghalensis* possesses asymmetry index of 62.58 with 2B in karyotype asymmetry (Table 1). A maximum of five nucleoli were observed through silver staining but no satellites were observed (Figure 1E). Two of the nucleoli are much larger than the other three nucleoli. Each of the larger nucleoli might have resulted from fusion of two or more nucleoli. It can be inferred that six or more chromosome satellites are present in this species.

The chromosome lengths differ from the smallest 2.5µm and gradually increased up to the largest 5.625µm (Table 1). The total length of whole chromosome complement was about 267.935µm (Table 1). The total length of the long arms of the diploid set was 167.675µm and that of short arms for diploid set was 100.26µm with their ratio generating an average karyotype asymmetry of 1.672. Generally, *C. benghalensis* (Addis Ababa) has predominantly metacentric and sub-metacentric type of chromosomes. The chromosomes were short to medium in size with an average length of 4.06µm.

C. diffusa from Ginchi

The variant of *C. diffusa* collected from Ginchi possessed 2*n*= 66 (Table 1 and Figure 1E). Based on centromeric ratio and chromosome length measurements , the karyotype (Figure 1F) consisted of fourteen metacentric pairs (pair numbers 1, 4, 6, 15, 16, 20, 21, 23, 25, 26, 30, 31, 32, 33), thirteen sub-metacentrics (pairs 2, 3, 5, 7, 8, 9, 10, 13, 17, 19, 24, 27, 28) and six sub-telocentrics (pairs 11, 12, 14, 18, 22, 29) with a formula of 28m + 26sm + 12st. The asymmetry index is 65.56 and belongs to 2B type of Stebbins karyotype classification (Table 1).

As shown in Figure 2(C and D), a total of six satellites and four nucleoli were observed from chromosome preparation and silver staining, respectively. Size of satellites varies with one pair being larger than the other two.

C. diffusa (Ginchi) has comparatively larger chromosomes than the other *Commelina* species included in this study with a length range from smallest 4.375µm to largest 11.25µm and total length of whole chromosome complement 507.53µm (Table 1). The total length of long arms and short arms for the diploid set was 332.745µm and 174.785µm respectively (Table 1). The average karyotype asymmetry is 1.904 and on the average the chromosomes can be classified under sub-metacentric. The chromosomes were medium to large in size with an average length of 7.69µm.

C. diffusa (Ginchi) has intra-chromosomal and inter-chromosomal asymmetry indices of 0.435 and 0.17035 respectively (Table 1). This variant also has a coefficient of variation in chromosome length of (17.035), ratio of centromeric gradient (53.0163) and dispersion index (9.031) (Table 1).

Table 1 Cytological analysis of Commelina L.

Species	Chromosome length (µm) Shortest: longest	ACL (µm)	2*n*	Basic number(*x*)	Ploidy level	A_1	A_2	C_v	C_G	DI	Karyotype Formula	Number of metaphase plates	A in
C. africana	4.53 – 7.5	5.89	30	15	2x	0.4345	0.1498	14.98	55.32	8.29	12m + 10sm + 8st	10	6
C. benghalensis	2.5 – 5.625	4.06	66	11	6*x*	0.3715	0.1582	15.82	57.831	0.3715	36m + 24sm + 6st	4	6
C.diffusa (Ginchi)	4.375 – 11.25	7.69	66	-	-	0.435	0.17035	17.035	53.0163	9.031	28m + 26sm + 12st	5	6
C.diffusa (Jimma)	2.5 – 4.58	3.47	30	15	2*x*	0.76717	0.13771	13.771	39.32	5.415	10m + 8sm + 12st	10	6
C. subulata	1.923 – 3.654	2.77	30	15	2*x*	0.3523	0.1891	18.91	67.45	0.3523	18m + 10sm + 2st	4	6

ACL: average chromosome length; 2*n*: somatic chromosome number; **A1:** intra-chromosomal asymmetry index; **A2:** inter-chromosomal asymmetry index; **C_v:** Coefficient of variation of chromosome length; **C_G:** ratio of centromeric gradient; **DI:** dispersion index **K:** percentage of chromosome with ratio=r>2;

L: ratio of largest to smallest chromosome; **M:** degree of asymmetry

***C. diffusa* from Jimma**

The material of this species collected from Jimma possessed 2*n*=2*x*= 30 (Table 1 and Figure 1G). According to centromeric ratio and chromosome length measurements, the karyotype (Figure 1H) has five metacentric pairs (pair numbers 1 to 5), 4 sub-metacentric pairs (pairs 6 to 9) and 6 sub-telocentric pairs (pair numbers 10 to 15) with a formula of 10m + 8sm + 12st. The asymmetry index is comparatively higher than the other studied *Commelina* species with a value of 69.497 and according to Stebbins karyotype asymmetry classification, *C. diffusa* (Jimma) belongs to 3A type (Table 1).

Even if no satellites were observed from the current plant material, a total of three interphase nucleoli were observed that possibly predict the number of NORs or satellites to be four (Figure 2F).

C. diffusa (Jimma) possesses the smallest chromosomes size next to *C. subulata* with a length range from shortest 2.5µm to longest 4.58µm and total length of whole 2*n* chromosome complement 104.22µm (Table 1). The total length of long arms for the diploid set was 72.43µm and total length of short arms for the diploid set was 31.79. The chromosomes range from very small to medium in size with an average length of 3.47µm and karyotype asymmetry of 2.28µm.

This particular variant has the highest value in terms of intra-chromosomal asymmetry index (0.76717). It also possesses the lowest inter-chromosomal asymmetry index (0.13771), coefficient of variation in chromosome length (13.771), ratio in centromeric gradient (39.32) and dispersion index (5.415) when compared to values from all other studied plants of the genus *Commelina*.

C. subulata

The experimental species collected from Ginchi indicated that *C. subulata* has a diploid number of 2*n* =2*x*= 30 (Table 1 and Figure 1I). As to arm ratio and chromosome length measurements, the karyotype (Figure 1J) consisted of nine metacentric pairs (pair numbers 1, 2, 3, 5, 8, 9, 10, 12, 13), five sub-metacentrics (4, 6, 11, 14, 15) and one pair of sub-telocentrics (7) with a karyotypic formula of 18m + 10sm + 2st. The species possesses the lowest value in asymmetry index (60.232), being the 2A type in Stebbins asymmetry classification (Table 1). Two interphase nucleoli were observed (Figure 2G), but no satellites were observed. One of the two nucleoli is larger than the other, which could result from fusion of two or more nucleoli; and it may be inferred that a total of four satellite chromosomes/ NORs are present.

Commelina subulata (Ginchi) has the smallest chromosome size than other species included in this study with a length range from smallest1.923µm to largest 3.654µm and the total length of whole chromosome complement is 83.154µm (Table 1). .

The total length of long arms and short arms for the diploid set was 50.085µm and 33.069µm, respectively (Table 1). The average karyotype asymmetry is 1.51 and the chromosomes, on the average arm ratio, can be considered as metacentric. The chromosomes are very small to medium in size with an average length of 2.77µm.

C. subulata possesses the lowest intra-chromosomal asymmetry index, highest ratio of centromeric gradient and dispersion index with a value of 0.3523, 12.755 and 67.45, respectively. The species also consisted of an inter-chromosomal asymmetry index (0.1891) and coefficient of variation in chromosome length (18.91) (Table 1).

Discussion

In the four species studied from the genus *commelina*, base numbers of *x*=11 and *x*=15 have been obtained. Base numbers *x*=11, 13 and 15 was also previously reported for *Commelina* [16, 17].The species *Commelina benghalensis* (2*n*=6*x*=66) was based on *x*=11 [18]. Others, *C. africana*, *C. diffusa* (Jimma) and *C. subulata* having chromosome number of (2*n*=2*x*=30) were based on *x*=15 base number. This result agrees with previous studies [15, 17, 19-24].

C. subulata of Ginchi has 2*n*=30 and this was in agreement with other reports from materials collected from Ethiopia [15] and Ghana [25]. Tetraploid species of *C. subulata* 2*n*=60 have also been reported for materials from India [26, 27]. *C. diffusa* from Ginchi has 2*n*=66 and this number is reported for the first time.

Chromosome numbers of *C. africana* collected from Addis Ababa and Sebeta were diploids 2*n*=30 and this was in agreement with previous reports [15]. Even if, the current count was diploid chromosome number, Morton [28] also found 2*n*=28 for materials from Ghana and polyploids (2*n*=60 and 2*n*= 120) were also reported [15]. *C. benghalensis* of Addis Ababa was found to be hexaploids (2*n*=6*x*=66) and this was in agreement with earlier report [25]. Both diploid 2*n*=22 and tetraploid (2*n*=44) cytotypes have also been reported for *C. benghalensis* from materials of Nigeria, China, India, Japan, Uganda, Ethiopia and Tanganyika [15, 18-21, 23, 25, 29, 30]. A diploid *C. diffusa* (2*n*=30) has been found for specimens collected from Jimma. This result was in agreement with other reports [15, 24].

Polyploid series of the West African *C. benghalensis*, *C. africana* and *Aneilema umbrsum* complexes are of autopolyploid origin based on observation of close similarity between polyploid and diploids with the absence of allied taxa which could have been involved in allopolyploidy [25].

A comparison made between the karyotypes of *C. diffusa* from Taiwan [23] and *C. diffusa* (Entoto mountain and Jimma) in the present study indicates that, although they share similar chromosome number (2*n*=30), they differ in karyotypic detail. The karyotype formula of the Taiwan specimen was reported with only two chromosomal groups, m and sm, (10m + 20sm), while in the present materials

three chromosomal groups (m, sm and st) with a formula of 16m+6sm+8st (Entoto) and 10m + 8sm + 12st (Jimma) were observed. Alam and Sharma reported variation in karyotypes among five populations of Indian *C. diffusa* having 2*n* = 30 [21]. Four different karyotype formula within 2*n*=30 chromosomes

from India were also reported previously [20]. Factors, other than the chromosomal heteromorphism, like deviation in techniques of chromosome preparation, condensation difference and measurement technique can also lead to karyotype diversity among reports by different workers. These factors hinder comparison between various karyotypes and for real comparison mean values for each measurement of individual chromosomes must be taken. Furthermore, karyotype comparison for the remaining species was not performed as all the chromosome report matching to the current chromosome number had not been found.

The chromosome report 2*n*=28 for the two species of *C. diffusa* and *C. africana* was probably associated with the uncommon aneuploids formed due to loss of chromosome number [15, 28]. But reduction in chromosome number can be associated with Robertsonian fusion [32] and translocation of all or most of its part followed by loss of the chromosome. High rate of prevalence of aneuploidy and polyploidy in the genus *Commelina* had reported [25].

Jones and Joplings reported the chromosome size of the genus *Commelina* as the smallest in the family [16]. Faden and Morton, on the other hand, confirmed the presence of medium to relatively large chromosome within the genus [17, 25]. In the present study, except for *C. subulata*, the size was predominantly medium, whereas *C. subulata* has an average chromosome smaller than the medium size limit (3µm).

The karyotype data of the present materials in the genus *Commelina* indicated the presence of three types of chromosomes with higher frequency of metacentric (m) and sub-metacentric (sm) types than sub-telocentrics (st). This was supported by earlier reports [17, 25]. However the ratio of each chromosome type considerably varies between each species.

A comparison made based on asymmetric index in the current study indicates that *C. africana* collected from Addis Ababa has same karyotype asymmetry with *C. diffusa* (Jimma) which possess 65.19% and 65.56%, respectively. *C. benghalensis* of the current study falls within 2B Stebbins category. This is in disagreement with the diploid material from Nigeria [30]. This might be associated with genetic difference that associated with difference in agro-ecology.

The present study revealed some degree of variation in chromosome length between the studied species with no major chromosomal difference among themselves. This may be associated with differences in degree of chromosome condensation between the metaphase spread of different species measured. Thus, in practice, it is difficult to draw taxonomic conclusions simply by comparing chromosome length between taxa [33] unless one compares chromosomes condensed to same degree.

Satellited chromosomes were observed frequently in Commelinaceae [25]. In the present study, satellites were observed in *C. africana* and *C. diffusa* (Ginchi). The reason why satellites were not observed in some of the species may be that the satellites are too small and escape easy cytological detection, or condense. All the satellites detected were located at the tip of the short arm of chromosome. Discrepancies in number of satellites reported for a particular taxon or population can be due to the inability to observe all the satellites because of variation in techniques of chromosome preparation, stages at the time of chromosome analysis and chromosomal polymorphism [34].

The maximum number of telophase nucleoli obtained through silver staining may correspond to the maximum number of satellites that are present in particular taxon [34]. Thus, even though all the satellites are not detected for various reasons discussed above, one can infer about the number of satellited chromosomes present in the taxon, if one is able to obtain the maximum number of nucleoli present. Accordingly it was assumed that the maximum number of satellites for *C. africana* will be four.

Nucleoli are formed at NORs of the satellited chromosomes. During the cell cycle, nucleoli disappear at late prophase and reform during telophase. It is less easy to observe the maximum number of nucleoli during telophase. As the cell cycle proceeds from telophase to interphase, nucleoli tend to fuse together, and thus their number in most of the interphase nuclei is usually less than their number in telophase nuclei. Though it is less frequently, it is possible that the nucleus enters interphase without all the nucleoli being fused, in which case the maximum number of nucleoli can also be observed in interphase nuclei. The maximum number of nucleoli observed, be at telophase or interphase, can be used to infer about the number of active NORs the plant possesses. Even numbers of maximum nucleoli are expected because NORs chromosomes occur as homologous pair (s). In case the highest number observed is odd number, one may take the next higher even number as the number of nucleoli for the organism. Usually when odd number is observed, at least one of the nucleoli is larger than the rest of the nucleoli indicating that the large nucleolus is the product of fusion of smaller nucleoli. In the present study, maximum number of nucleoli observed for *C. africana* (Addis Ababa) is three, and for *C. africana* (sebeta) and *C. diffusa* (Entoto) is four each which allow to make an inference that they all have 4 NORs (satellite chromosomes).

The number of nucleoli observed for *C. benghalensis* is five but two are very large relative to the other three nucleoli. The large ones are possibly fusion products of two or more small nucleoli. There are at least three pairs or more satellited chromosomes in this species.

Conclusions

The present study has revealed and confirmed chromosome number, ploidy level, karyotype and nucleolus numbers of six species of Commelinaceae which were collected from different localities of central and south western part of the country. Accordingly, this study showed that basic chromosome number for three species of *Commelina* (*C. diffusa, C. africana* and *C. subulata*) is *x*=15 and 2*n*=30. The chromosomes of these species are predominantly of m and sm types. *C. benghalensis* (2*n*=6*x*=66) was also found to have *x*=11. The basic chromosome number for *C. diffusa* (Ginchi), 2*n*=66 was different from previous reports and hence it could be another cytotype for the species. Variation in karyotype formula is also observed within species of *C. africana* and *C. diffusa* collected from different localities. *C. diffusa* (Ginchi) is vigour, longer with distinct morphology than the other diploid species. This is the first work to present karyotypes, satellite chromosomes and nucleoli of the Ethiopian Commelinaceae. Nevertheless an integrated data from karyotype, molecular study of chloroplast genome and evolution through considering more representative species is necessary to infer phylogenetic relationship among the taxa.

Methods

Four species of the genus *Commelina* which were collected from different localities of Central and Southwestern part of Ethiopia were analyzed cytologically. Chromosome study including numbers and morphology (karyotypes) was done on C-metaphase chromosomes of the root tip meristematic cells.

Pretreatment

To obtain clean roots for chromosome preparation, stems were harvested from potted plants and allowed to stand submerged in water. In a few days, roots emerged from the nodes of the submerged stems and harvested for metaphase arrest of mitosis. Hence, roots were immersed in 8-hydroxyquiniline (0.002M) for 3 to 5 hrs, fixed in 3:1 (v/v) of ethanol and glacial acetic acid 1-24 hr at about 4^{o}C.

Maceration

Roots from fixative were rinsed several times in distilled water and macerated in a solution of pectinase and cellulase in a water bath at 37^{o}C for about 1 hr or more. The process was stopped when the tips of the root started detaching from the root with or without agitation of the vial. Then, enzyme solution was carefully pipetted out and transferred to a watch glass or a petridish after rinsing.

Air dry slide preparation

One or more of the macerated root tips (depending upon the size) were pipetted on a clean slide and fresh fixative (3:1, ethanol: acetic acid) were added to the root tips, and the tips were mashed quickly with flat ended mounted needle. The slide was then allowed to air dry at room temperature and stored until needed for staining.

Slide staining

Slides with good preparations were screened under phase contrast microscope and good preparations were screened under phase contrast microscope before staining. The promising preparation was stained in Giemsa stain in Sorenson phosphate buffer solution (PH 6.8). When correct contrast of staining was obtained, the slides were rinsed in distilled water, and allowed to air-dry at room temperature at least for 24 hrs. The preparation was made permanent by mounting in depex mountant and the gum was allowed to set for several days following by examining under camera fitted microscope. Then, enlarged photomicrograph prints were made accordingly.

Silver staining

Slide preparation was similar with the above with the exclusion of treatment of 8-hydroxyquiniline. The latter treatment was omitted in order to obtain telophase cells during which the maximum number of nucleoli may be observed. Silver staining technique was similar to the work of Dagne and Heneen [35].

Karyotype analysis

The printed pictures of chromosomes were scanned into computer and the lengths of the whole chromosomes and their arms were measured in terms of pixel per cm using micro measure computer software version 3.3. Accordingly, the arm ratio of the chromosomes was calculated by dividing the length of the long arm to that of short arm.

Karyotypes were constructed by cutting and arranging the putative homologous chromosomes into pairs based on arm ratio(*r*) and chromosome size using the Smart Type software version 0.8. Chromosomes were categorized into chromosome types based on arm ratio (*r*) according to [36] with slight modification. In the present case the term metacentric chromosome was used to include both M and m types with r =1.0-1.7. Sub-metacentric was used as synonymous with sub-median chromosome of r= 1.7-3.0 and st is similar to sub-telocentric when r=3.0-7.0.

After accurate measurements of karyotyped chromosomes were obtained, intra-chromosomal asymmetry (A_1) and inter-chromosomal asymmetry (A_2) indices have been calculated [37]. Moreover, DI (dispersion index) of chromosomes was also calculated [38].

Measurements like intra chromosomal (A_1), inter-chromosomal (A_2), dispersion indices (DI) and Stebbins asymmetry depends on both chromosome size and centromeric position for estimating karyotype asymmetry of chromosomes. But asymmetry index depends only on centromeric position [39].

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The data used for current study can be provided on request.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

SG prepared the publication manuscript and all experimental works. GK, BG, GM and DG review all the work and incorporated necessary comments for better output.

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Figures

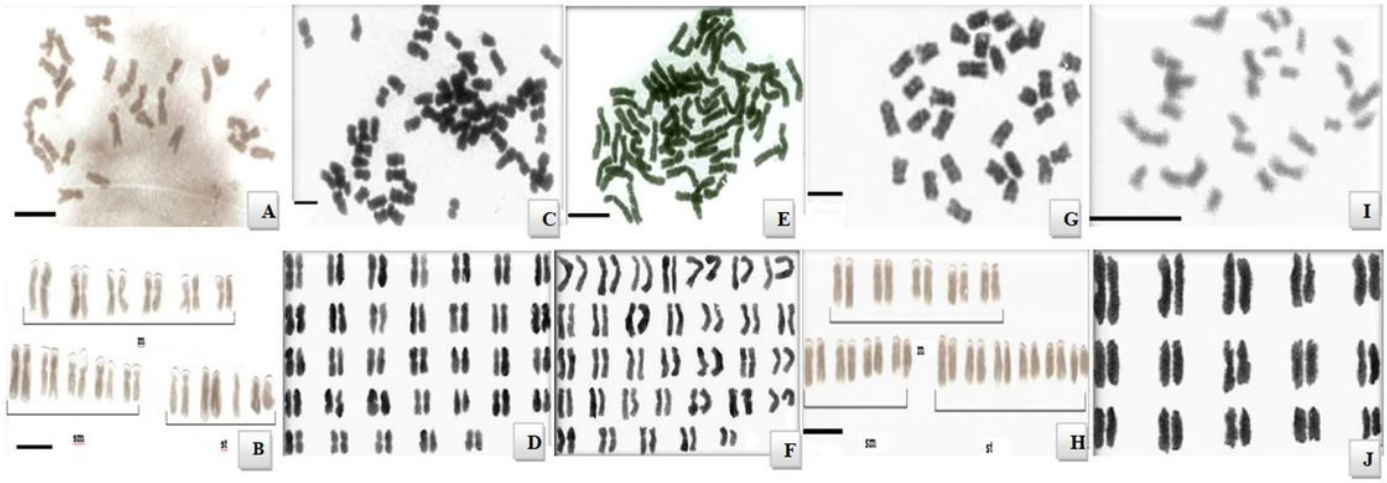
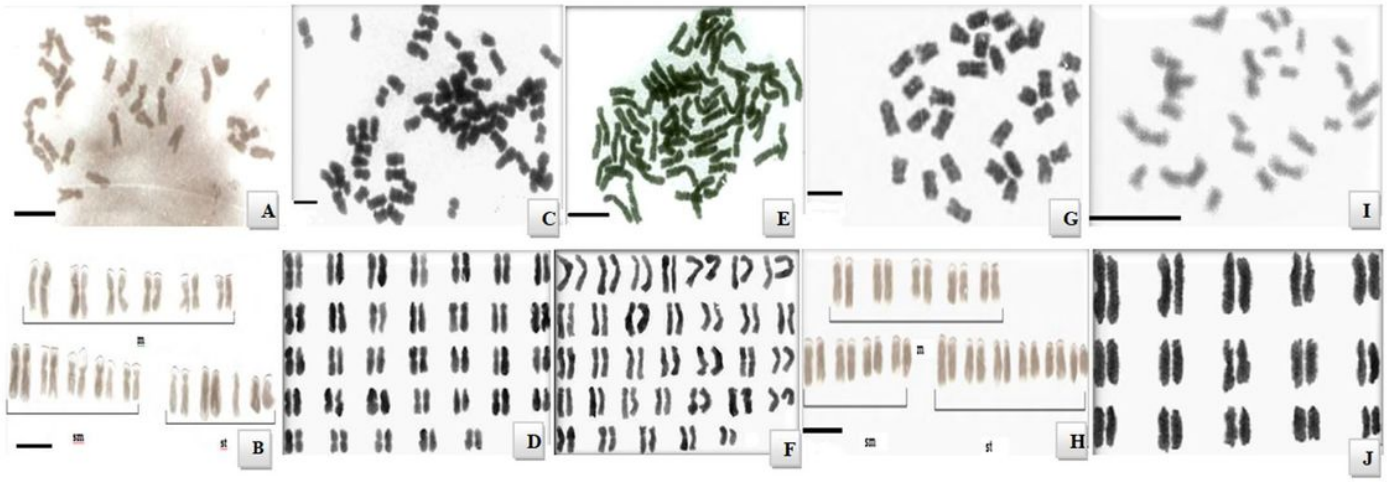


Figure 1

Somatic metaphase spread and karyotype of the genus Commelina L.; C. africana (A & B), C. benghalensis (C &D), C. diffusa (Ginchi) (E & F), C. diffusa (Jimma) (G &H), C. subulata (I&J); A, C, E, G & I reveal the metaphase spread where as the remaining images are karyotypes; horizontal bar indicates the scale at 5 µm for C. benghalensis & C. subulata where as the scale bar for C. africana, C. diffusa (Ginchi) & C. diffusa (Jimma) is 10 µm



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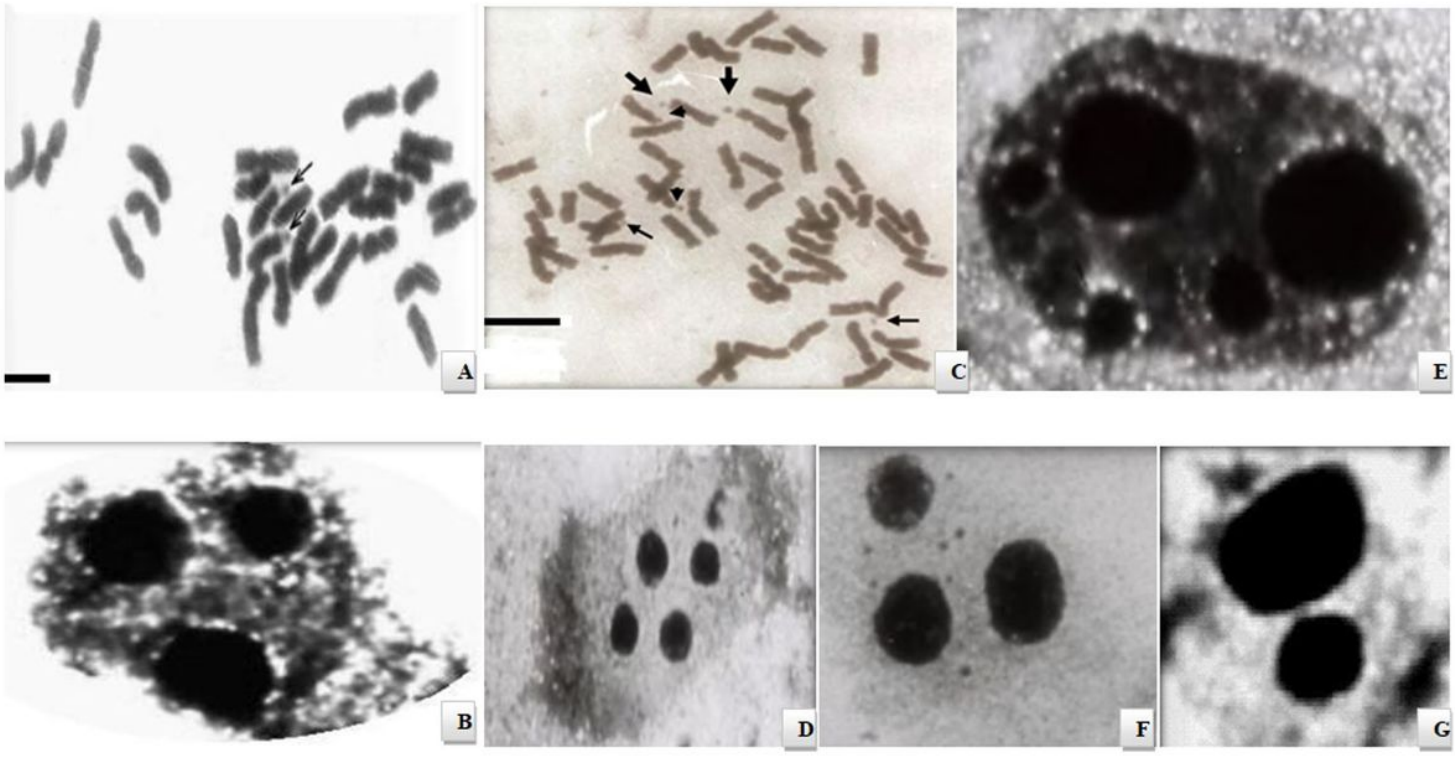
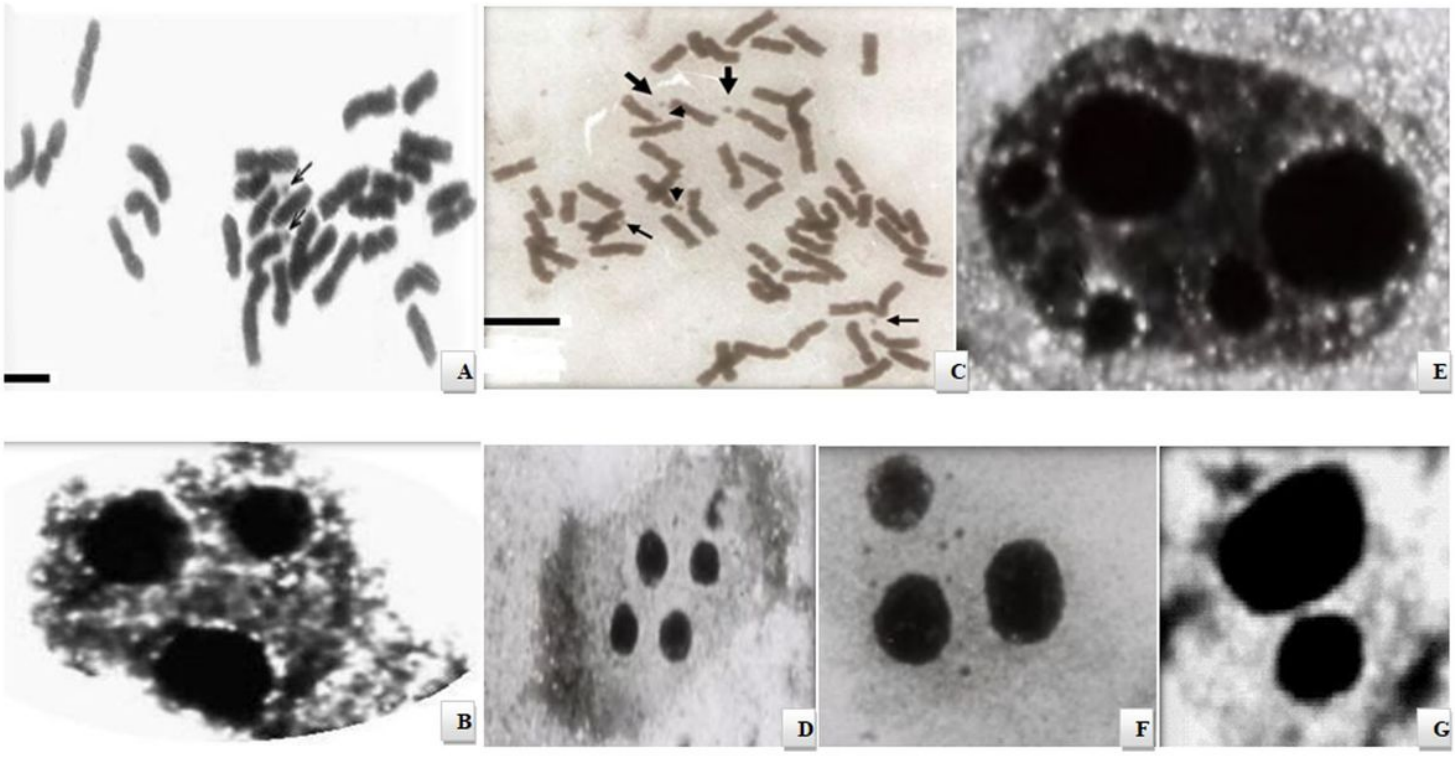


Figure 2

NORs and interphase nucleoli of Commelina L. A. two satellites of C. africana B. Three nucleoli of C. africana C. five NORs of C. diffusa from Ginchi; D, E, F. & G indicates nucleoli of C. diffusa (Ginchi), C. benghalensis, C. diffusa (jimma) and C. subulata respectively. Arraws indicate the specific position of satellites



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