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## Karyotype variability of the genus *Colocasia* (Araceae) of Assam, North East India

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**Abstract.** Cytological study in four species of *Colocasia* (Araceae) of Assam showed a variation of chromosome numbers. Basic chromosome number of the species was reported as  $n = 14$ . Dominance of metacentric chromosomes in all the four species and symmetric karyotypes indicate the primitive evolutionary status of the species. Analysis of chromosome asymmetry indices indicate the karyotype homogeneity. Deviation of basic chromosome numbers in *Colocasia manii* Hook. f. and *Colocasia fallax* Schott. reflects the possible existence of aneuploidy. Presence of secondary constriction indicates the chromosomal plasticity.

**Keywords:** asymmetry indices, chromosome number, *Colocasia*, karyotype.

### INTRODUCTION

Araceae is a diverse plant group that comprises of 114 genera and 3750 species (Petruzzello 2018). Most of the genera under the family Araceae are predominantly found in tropical Asia (Grayum, 1990). The genus *Colocasia* under the family Araceae is predominantly found in Asia and South-East Asia is the primary centre of origin of this genus (Plucknett 1979). Large number of species and ecotypes of *Colocasia* have been reported from North Eastern part of India (Anbazhagan et al. 2015; Angami et al. 2015). The genus comprises of tropical, evergreen, perennial herb and are important sources of food and medicine. *Colocasia* has been used as an integral part of cuisine by various communities of Assam, India since time immemorial (Baro et al. 2023). The genus is highly polymorphic. Li and Boyce (2010) reported twenty (20) species of *Colocasia* over the world out of which six (6) species are found in Assam (Menla et al. 2019). Several species and ecotypes under this genus have been reported by Ahmed et al. (2020) on the basis of morphological characters of corm and aerial parts of the plant. Devaraju et al. (2023) reported considerable degree of variability in *Colocasia esculenta* (L.) Schott. Cytological investigations have also been reported by some workers on few species of the genus *Colocasia* (Cao and Long 2004; Senavongse et al. 2018) from different parts of the world.

Chromosome number and genome size are important cytological characters that significantly influence various organismal traits. Karyo-morphological information can open a new direction for evaluating evolutionary status of different species of the genus. The chromosome number in *Colocasia* is reported as  $2n=28$  and  $42$  by Wulansari et al. (2021). Variations of chromosome numbers have also been reported in the genus *Colocasia* (Saensouk et al. 2019). Presence of intra-specific variation of chromosome numbers in *Colocasia* species had been reported by Coates (1988). Chromosome diversity at the intra and inter-specific level is necessary for adaptation and survival of species in changing environmental conditions. Chromosome study and karyotype analysis have been described as important parameters to estimate the inter and intra-specific diversity of various species within a genus (Stebbins 1971; Young et al. 2012) and can be a major aid for distinguishing taxonomic groups, deducing taxonomic relatedness and evolutionary status (Lavania 1985; Lorenzo and Eroglu 2013). Chromosomal polymorphism, potent promoters of reproductive isolation and speciation can be further correlated with differences in morphological parameters. The aim of this study is to establish the cytotaxonomic relationship among four (4) different species of *Colocasia* found in Assam.

## MATERIALS AND METHODS

Four species of *Colocasia* Schott viz. *Colocasia manii* Hook. f., *Colocasia esculenta* (L.) Schott., *Colocasia fallax* Schott. and *Colocasia gigantea* (Blume) Hook. f. was collected from various locations of Assam, India and were maintained under optimal conditions for root initiation. Newly emerging healthy roots of 5-7 mm size were treated with 0.008 M (aq.) 8-Hydroxyquinoline (oxine) for one (1) hour (Tlaskal 1979; Nair 2016). The root tips were then washed thoroughly with distilled water, treated with 0.075 M KCl and were macerated in a mixture 1.5 % acetocarmine and 1 N HCl in 9:1 (v/v) ratio. The slides were observed under microscope and well separated metaphase stages were photographed at magnification of  $100\times \times 45\times$  and proceeded with karyotyping using the software assisted imaging application. Length of long arm (L), length of short arm (S), length of chromosome (CL), total chromosome length of diploid complement (TCL), arm ratio (AR), relative length percentage (RL%), centromeric index (CI) were considered for the characterization of the karyotypes. The nomenclature of the chromosome type and morphology was done following the standard system proposed.

Chromosome asymmetry indices was measured by considering TF% (Huziwara 1962), Stebbins' classes A-C (Stebbins 1971), Ask% (Arano 1963), karyotype asymmetry A (Watanabe et al. 1999), intrachromosomal and asymmetry index ( $A_1$ ) and interchromosomal asymmetry index ( $A_2$ ) (Zarco 1986), co-efficient of variation of chromosome length ( $CV_{CL}$ ), co-efficient of variation of centromeric index ( $CV_{CI}$ ) and asymmetry index (AI) (Paszko 2006).

## RESULTS AND DISCUSSION

The genus showed variations in chromosome number and morphology. Detailed chromosome and karyotype parameters of four species of *Colocasia* are shown in Table 1, Figure 1.

### *Colocasia manii* Hook.f.

In *Colocasia manii* Hook. f. somatic number was  $2n=30$  with karyotype formula  $2n=30=24m+4sm+2st$ . Range of single chromosome length was  $5.71-21.42\ \mu\text{m}$ . Total chromosome length was  $343.57\ \mu\text{m}$ , relative chromosome length ranges from 2.07 to  $6.23\ \mu\text{m}$ . The arm ratio and centromeric index were recorded as 1.00–2.72 and 0.36–0.50 respectively.

### *Colocasia fallax* Schott

*Colocasia fallax* Schott showed chromosome number  $2n=24$  and  $2n=28$  with somatic karyotype formula  $2n=24=18m+4sm+2st$  and  $2n=28=2M+18m+8sm$  respectively. The genotype with chromosome number  $2n=24$  has total chromosome length (TCL)  $683.48\ \mu\text{m}$  with relative chromosome length ranges from  $2.18-7.15\ \mu\text{m}$ . Range of arm ratio was 1.00–2.16 and centromeric index was recorded as 0.31–0.50. While in the genotype with  $2n=28$  chromosome number, the total chromosome length (TCL) was recorded as  $635.12\ \mu\text{m}$ . The relative chromosome length, range of arm ratio and centromeric index were  $2.20-4.95\ \mu\text{m}$ , 1.02–3.25 and 0.31–0.49 respectively.

### *C. esculenta* (L.) Schott

In *C. esculenta* (L.) Schott somatic chromosome number was recorded as  $2n=28$  with somatic karyotype formula  $2n=28=22m+6sm$ . Range of single chromosome length was recorded as  $11.00-34.66\ \mu\text{m}$ . Total chromosome length was  $618.99\ \mu\text{m}$ . The relative length of chro-

**Table 1.** Chromosome parameters of four genotypes of the genus *Colocasias*.

| Species                            | Chromosome number (2n) | Karyotype formula | Length of short arm (μm) | Length of long arm (μm) | Single chromosome length (μm) | Relative length of chromosome (%) | Arm Ratio (AR) | Centromeric index (CI) | Karyotype Symmetry/asymmetry (S/AI) Index |
|------------------------------------|------------------------|-------------------|--------------------------|-------------------------|-------------------------------|-----------------------------------|----------------|------------------------|---|
| <i>Colocasias manii</i> Hook.f.    | 2n=30                  | 24m+4sm+2st       | 2.14–9.64                | 3.57–11.78              | 5.71–21.42                    | 2.07–6.23                         | 1.00–2.72      | 0.36–0.50              | 1.3                                       |
| <i>C. fallax</i> Schott            | 2n= 24                 | 18m+4sm+2st       | 4.98–21.95               | 8.98–25.94              | 13.96–47.89                   | 2.18–7.15                         | 1.00–2.16      | 0.31–0.50              | 1.4                                       |
|                                    | 2n= 28                 | 2M+18m+8sm        | 1.92–7.91                | 3.33–8.52               | 6.9–15.9                      | 2.20–4.95                         | 1.02–3.25      | 0.31–0.49              | 1.3                                       |
| <i>C. esculenta</i> (L.) Schott.   | 2n=28                  | 22m+6sm           | 5.00–12.66               | 6.00–22.00              | 11.00–34.66                   | 1.88–5.37                         | 1.00–2.07      | 0.31–0.52              | 1.2                                       |
|                                    | 2n=28                  | 2M+22m+4sm        | 5.60–12.80               | 6.56–13.43              | 12.16–26.22                   | 2.57–5.40                         | 1.00–1.57      | 0.38–0.50              | 1.1                                       |
| <i>C. gigantea</i> (Blume) Hook.f. | 2n=30                  | 4M +20m+ 4sm+2st  | 3.32–8.46                | 3.80–13.39              | 7.21–21.83                    | 2.01–5.53                         | 1.00–3.75      | 0.211–0.483            | 1.3                                       |
|                                    | 2n=32                  | 2M+22m+4sm+4st    | 2.84–5.72                | 3.21–10.20              | 6.06–15.91                    | 2.05–4.704                        | 1.00–3.50      | 0.222–0.51             | 1.4                                       |

mosome, arm ratio was recorded as 1.88–5.37, 1.00–2.07 respectively and centromeric index was 0.31–0.52.

#### *C. gigantea* (Blume) Hook.f.

Somatic number was recorded as 2n=28, 2n=30 and 2n=32 with karyotype formula 2n=28=2M+22m+4sm, 2n=30=4M+20m+4sm+2st and 2n=32=2M+22m+4sm+4st respectively. In genotype with 2n=28, single chromosome length ranges from 12.16 to 26.22 μm, total chromosome length was 567.49 μm; relative chromosome length ranges from 2.57 to 5.40; arm ratio and centromere index were recorded as 1.00–1.57 and 0.38–0.50 respectively.

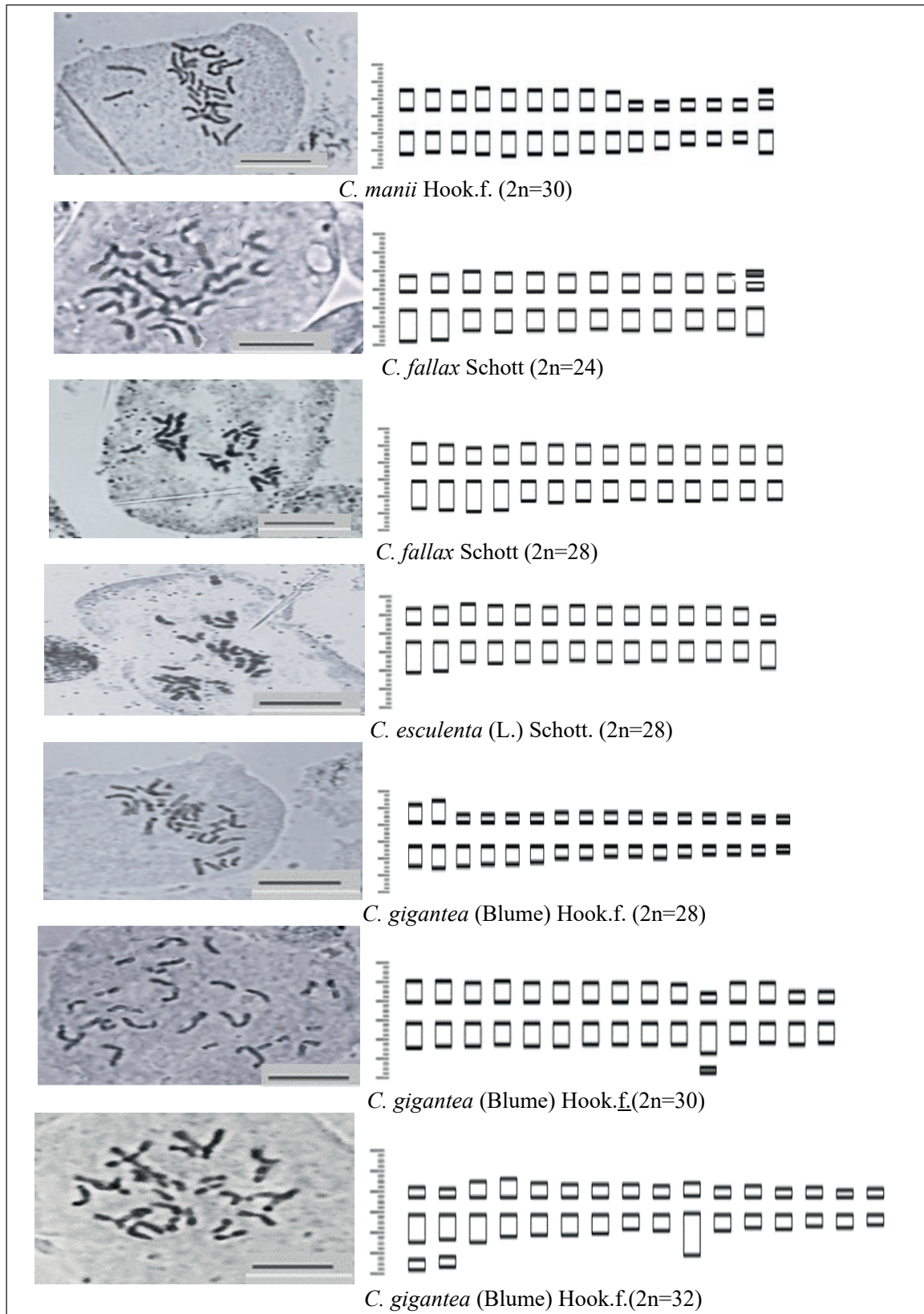
For genotype with 2n=30, total chromosome length was recorded as 525.01 μm, relative chromosome length, arm ratio and centromeric index were found as 2.01–5.53, 1.00–3.75 and 0.211–0.483 respectively. In genotype 2n=32 the total chromosome length was 632.51 μm. Relative length of chromosome was 2.05–4.704 μm, arm ratio and centromeric index were recorded as 1.00–3.50 and 0.222–0.51 respectively.

Chromosome symmetry/asymmetry index (S/AI) for all seven (7) chromosomes complement showed symmetric karyotypes with the range from 1.1 to 1.4 (Table 1). All seven chromosome complements showed chromosome symmetry.

#### Karyotype asymmetry indices

Karyotype asymmetry indices of all the seven karyotypes and scattered diagrams are presented in Table 2, Figs 2, 3 & 4.

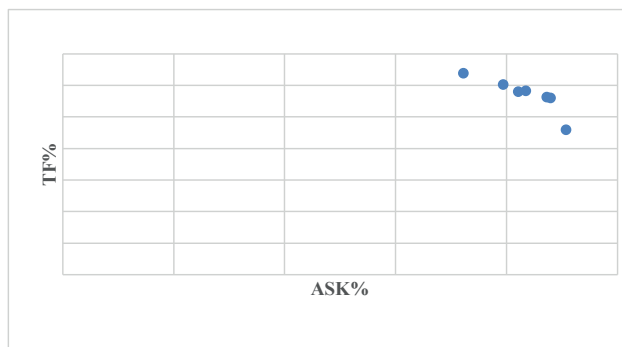
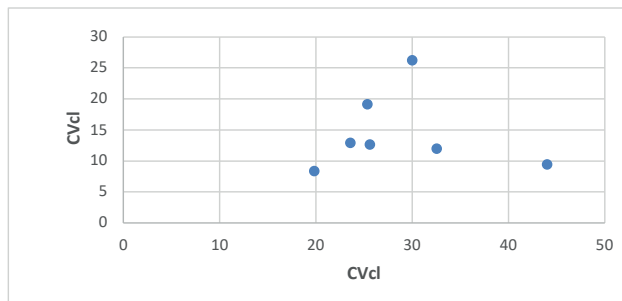
Among the karyotypes the highest TF% was recorded for *C. gigantea* (Blume) Hook.f. 2n=28 and lowest was also in the same genotype with chromosome complement 2n= 32. TF% and AsK% showed perfect negative correlation for all seven karyotypes. Except for the chromosome complements 2n=30 and 2n=32 of *C. gigantea* (Blume) Hook.f. TF% values fall in 41.08–45.39 which indicates symmetric karyotype. Lower value of TF% in chromosome complements 2n=30 and 2n=32 of *C. gigantea* (Blume) Hook.f. were recorded as 39.69 and 39.20 respectively and reflected karyotype asymmetry for these two complements. A1 and A2 values ranges from 0.15–0.30 and 0.19–0.44 respectively. A1 and A2 showed negative correlation for the complements 2n=32 of *C. gigantea*, (Blume) Hook.f. 2n=24 and 2n=28 of *C. fallax* Schott. and 2n=28 of *C. esculenta* (L.) Schott. While for 2n=28 of *C. gigantea* (Blume) Hook.f. A1 showed positive correlation with A2. CVcl and CVci values fall in the range of 19.82–44.02 and 9.45–20.73 respectively. Highest value of



**Figure 1.** Karyotypes and ideogram of *Colocasia* genotypes of Assam.

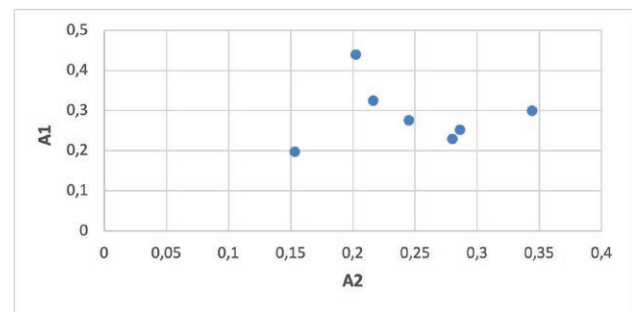
**Table 2.** Karyotype asymmetry indices of four Species of *Colocasia* species.

| Karyotype indices | <i>C. manii</i> Hook.<br>f. | <i>C. fallax</i> Schott |       | <i>C. esculenta</i> (L.)<br>Schott | <i>C. gigantea</i> (Blume) Hook. f. |       |       |
|-------------------|-----------------------------|-------------------------|-------|------------------------------------|-------------------------------------|-------|-------|
|                   | 2n =30                      | 2n=24                   | 2n=28 | 2n=28                              | 2n=28                               | 2n=30 | 2n=32 |
| TF%               | 43.99                       | 43.64                   | 41.08 | 41.73                              | 45.39                               | 39.69 | 39.20 |
| ASK%              | 55.99                       | 56.35                   | 58.01 | 58.26                              | 46.0                                | 60.30 | 60.79 |
| A                 | 0.118                       | 0.129                   | 0.17  | 0.149                              | 0.45                                | 0.190 | 0.199 |
| A <sub>1</sub>    | 0.20                        | 0.216                   | 0.28  | 0.245                              | 0.15                                | 0.28  | 0.303 |
| A <sub>2</sub>    | 0.44                        | 0.325                   | 0.23  | 0.276                              | 0.19                                | 0.253 | 0.230 |
| CV <sub>CL</sub>  | 44.02                       | 32.55                   | 23.58 | 25.62                              | 19.82                               | 25.33 | 23.04 |
| CV <sub>CI</sub>  | 9.45                        | 11.99                   | 12.91 | 12.67                              | 8.36                                | 19.17 | 20.73 |
| AI                | 26.56                       | 27.13                   | 28.21 | 21.93                              | 23.69                               | 23.21 | 21.14 |
| Stebbins' Type    | 1C                          | 2B                      | 1B    | 2B                                 | 1B                                  | 1B    | 1B    |

**Figure 2.** Scattered diagram for AsK % against TF %.**Figure 3.** Scattered diagram for CVCL against CVCI.

CVcl & CVci were recorded in 2n=30 of *C. manii* Hook. f. and 2n=32 of *C. gigantea* (Blume) Hook.f. The CVcl and CVci was evident for positive correlation and their higher range of values indicated the heterogeneous karyotypes. AI values range from 21.14 –28.21.

Under the present investigation, *C. manii* Hook. f. (2n=30); *C. fallax* Schott. (2n=24 and 2n=28), *C. esculenta* (L.) Schott. (2n=28) and *C. gigantea* (Blume) Hook.f.

**Figure 4.** Scattered diagram for A2 against A1.

(2n=28) showed asymmetric karyotype with respect to TF%, A1, A2 and AI; but showed symmetric karyotype with accordance to AsK%, A, CVcl for the complement 2n=28 of *C. fallax* Schott. and 2n=28 of *C. esculenta* (L.) Schott. The complements 2n=30 and 2n=32 of *C. gigantea* (Blume) Hook.f. showed symmetric karyotype for the indices TF%, CVcl, AI and asymmetric karyotype for the indices AsK%, A, A1, A2 and CVci. All the genotypes with different chromosome complements showed Stebbins' asymmetry class 2B and 1B except for *Colocasia manii* Hook. f. which exhibited 1C.

Detail chromosome morphology and karyotype analysis is the potential source to establish the relationship among the genotypes and also to find out the divergence among the genotypes. Diversity of chromosome numbers (2n=26, 28, 36, 38, 42 and 56) in the genus *Colocasia* and also the presence of polyploidy cytotype has been reported (Yang 2003; Wang et al. 2017). Das et al. (2015) also reported ploidy level in *Colocasia* species with chromosome number 2n=42 (triploid). Under the present investigation the basic chromosome number n = 14 was found in all the four (4) species. Chair



et al. (2016) also reported genomic number  $n = 14$  in the genus *Colocasia*. However intraspecific variation of chromosome numbers was found in *C. fallax* Schott ( $2n=24$  &  $2n=28$ ) and in *C. gigantea* (Blume) Hook.f. ( $2n=28$ ,  $2n=30$  &  $2n=32$ ). Wang et al. (2017) reported inter and intraspecific chromosomal variation in five species of *Colocasia* with chromosome count  $2n= 26, 28, 38, 42$ , and  $56$ . Variation in somatic chromosome numbers in mitotic cells of many angiosperm species under the genus *Phalaris* (Poaceae) was reported by Winterfeld et al. (2018). Karyotype analysis may be good tools for identification of intra and inter-species variation but under the present investigation, the diversity in chromosome number within a species became a hindrance to establish the karyotype evolution with related taxa.

### CONCLUSION

Karyotype analysis of *Colocasia* genotypes under present investigation showed basic chromosome number  $n=14$ . The prevalence of more metacentric chromosomes indicates primitiveness and chromosome symmetry. TF % and uniform Stebbins' chromosome type of the species also indicate karyotype symmetry and karyotype homogeneity implying primitiveness of the genus. Lower and almost uniform values of AI,  $CV_{CL}$ ,  $CV_{CI}$ , A1 and A2 also reflect karyotype homogeneity. Deviation of basic chromosome number from  $n=14$  in some genotypes under two species viz. *C. fallax* Schott. and *C. gigantea* (Blume) Hook.f. gives an indication towards the occurrence of aneuploidy and chromosomal plasticity. Aneuploid in the species may widen the genetic variations which may lead to the formation of different diagnostic morphological and floral characters in the species. Presence of secondary constriction in the certain genotypes indicates high chromosomal plasticity and their potential relevance to chromosome evolution. Variation of chromosome number which may be due to aneuploidy or euploidy may cause variety of phenotypic changes in the species including plant architecture. These variations may cause dosage imbalance of gene on the affected chromosomes that may alter the phenotypic alteration of the species. In *Colocasia* under present investigation, variation of chromosome number within the species may contribute towards the establishment of new species.

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