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REPORT

Karyomorphological studies in three species of *Argyreia* Lour. (Convolvulaceae) from India

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Abstract
In this study, karyotypes of three taxa in the genus *Argyreia* Lour. were examined. The chromosome counts for *A. osyrensis* and *A. setosa* were reported for the first time while somatic chromosome count of *A. cuneata* was confirmed. The three taxa studied were diploid with $2n = 2x = 30$. Highest total chromosome length in *Argyreia osyrensis* was 54.25 µm with mean chromosome length (MCL) of 3.62 µm. The lowest in *Argyreia setosa* as 46.04 µm with the mean chromosome length of 3.07 µm. The karyotypes of the species were examined on the grounds of various karyotype asymmetry indices. Two of them, *A. cuneata* and *A. osyrensis* fell in the 3A while *A. setosa* belonged to the 4A category of Stebbins' classification.

Keywords *Argyreia* · Assymetry indices · Chromosome count · Convolvulaceae · Karyotype

Introduction

Argyreia Lour. is taxonomically complex genus of the family Convolvulaceae. It is native to continental Asia, with a majority of the species distributed in Southeast Asia, the Indian subcontinent and China. India comprises 40 taxa which includes 37 species and 3 varieties [15] among which 17 species are endemic to India (Table 1). Hooker [2] recognized two sections in *Argyreia* based on sepal morphology as *Pomifera* C.B. Clarke and *Euargyreia* C.B. Clarke. The three species investigated in this report belong to sect. *Euargyreia* (*Argyreia*). Ooststroom [7] in Flora Malesiana divided *Argyreia* into two sections, viz. *Ptyxanthus* G. Don and *Schizanthus* G. Don on the basis of lobed or entire corolla and included or exserted reproductive whorls. *A. cuneata* and *A. setosa* belongs to sect. *Ptyxanthus* while *A. osyrensis* belong to sect. *Schizanthus*. But the above said sectional names are not correct according to the ICN. As in case of sect. *Euargyreia*, infrageneric names starting with prefix 'Eu' are not validly published and should be named as sect. *Argyreia*. Another case of sect. *Schizanthus*, it includes the type of *Argyreia* hence must be called as sect. *Argyreia* [15, 17]. *Argyreia cuneata* (Willd.) Ker Gawl. is unique in being a medium-sized erect shrub; purple–carmine red colored flowers with included stamens (Fig. 1a). *A. osyrensis* (Roth) Choisy and *A. setosa* (Roxb.) Sweet are climbing, twinning shrubs with exserted stamens or stamens up to corolla mouth (Fig. 1c, e). The studied species are distributed in the Indian subcontinent and Southeast Asia.

Sampathkumar and Ayyangar [10], who have intensively studied the karyophyletic affinities among the members of Convolvulaceae and suggest that the similarity in overall chromosome morphology, somatic chromosome counts and almost similar chromosome sizes in the genera *Argyreia*, *Rivea* and *Stictocardia* may represent a common line of evolution. *Argyreia* is the second most species-rich genus of Indian Convolvulaceae with immense ornamental potential and considerably longer chromosome size. Even though, cytotaxonomic records for the genus are very few [1, 3, 6, 9–13, 18] and are mainly confined to somatic counts. The only meiotically screened species *A. bella* exhibits basic number, $x = 14$ [12]. Among the studied taxa, seven species have been recorded with somatic chromosome count $2n = 2x = 30$ chromosomes while two species, *A. argentea* and *A. bella*, had $2n = 2x = 28$ chromosomes (Table 1). There are no chromosome reports for the Southeast Asian endemic species of *Argyreia* considered to be species rich centre for the genus.

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Table 1 *Argyreia* species in India, their distribution and somatic counts (2*n*)

Sr. no.	Taxa	2*n*	Reference	Geographical distribution
1.	**Argyreia arakuensis* N.P. Balakr.	–	–	Andhra Pradesh (Araku valley)
2.	*Argyreia argentea* (Roxb.) Sweet	28	[13]	Assam, Madhya Pradesh, Meghalaya, Tripura, Tamil Nadu
3.	*Argyreia atropurpurea* (Wall.) Raizada	–	–	Sikkim, West Bengal
4.	*Argyreia bella* (C.B.Clarke) Raizada	28	[11]	Andhra Pradesh, Bihar, Jharkhand, Madhya Pradesh, Orissa
5.	**Argyreia boseana* Santapau & V. Patel	–	–	Maharashtra
6.	**Argyreia bracteata* Choisy	–	–	Kerala, Tamil Nadu
7.	*Argyreia capitiformis* (Poir.) Ooststr.	–	–	Assam, Manipur, Meghalaya, West Bengal
8.	**Argyreia coonoorensis* W.W.Sm. & Ramaswami	–	–	Tamil Nadu- Coonoor- Nilghiri
9.	*Argyreia cuneata* (Willd.) Ker Gawl.	30	[12], Present Communication	Andhra Pradesh, Karnataka, Kerala, Maharashtra
10.	*Argyreia cymosa* (Roxb.) Sweet	–	–	Andhra Pradesh, Madhya Pradesh, Maharashtra, Tamil Nadu, West Bengal
11.	**Argyreia daltonii* C.B.Clarke	–	–	Andhra Pradesh, Jharkhand, Orissa, Tamil Nadu
12.	*Argyreia elliptica* (Roth ex Roem. & Schult.) Choisy	–	–	Maharashtra, Karnataka, Kerala, Tamil Nadu
13.	**Argyreia fulgens* Choisy	–	–	Kerala
14	*Argyreia hirsuta* Wight & Arn.	30	[12]	Karnataka, Kerala, Tamil Nadu
15.	*Argyreia hirsutissima* (C.B.Clarke) Raizada	–	–	Nagaland, Sikkim
16.	*Argyreia hookeri* C.B. Clarke	30	[9]	Andaman Islands, Karnataka, Kerala, Maharashtra, Sikkim, Tamil Nadu, West Bengal
17.	**Argyreia involucarata* C.B. Clarke	–	–	Bihar, Goa, Maharashtra, Karnataka, Tamil Nadu
18.	**Argyreia involucrata var. inaequalis* C.B.Clarke	–	–	Maharashtra, Karnataka, Kerala
19.	*Argyreia kleiniana* (Roem. & Schult.) Raizada	–	–	Kerala, Tamil Nadu
20.	**Argyreia kondaparthiensis* P.Daniel & Vajr.	–	–	Andhra Pradesh, Tamil Nadu
21.	**Argyreia lawii* C.B.Clarke	–	–	Telangana
22.	**Argyreia leschenaultii* Choisy	–	–	Andhra Pradesh, Kerala, Karnataka
23.	*Argyreia mastersii* (Prain) Raizada	–	–	Nagaland, Mizoram
24.	*Argyreia mollis* (Burm.f.) Choisy	–	–	Andaman Islands
25.	**Argyreia nellygherya* Choisy	–	–	Tamil Nadu—Nilgiri
26.	*Argyreia nervosa* (Burm.f.) Bojer	30	[18]	Throughout India (Cultivated as ornamental)
27.	*Argyreia osyrensis* (Roth) Choisy	30	Present communication	Jharkhand, Karnataka, Tamil Nadu
28.	**Argyreia pilosa* Wight & Arn.	–	–	Maharashtra, Karnataka
29.	*Argyreia roxburghii* (Sweet) Choisy	–	–	Arunachal Pradesh, Assam, Meghalaya, West Bengal
30.	**Argyreia sericea* Dalzell	–	–	Gujarat, Madhya Pradesh, Maharashtra, Kerala, Rajasthan, Tamil Nadu
31.	*Argyreia setosa* (Roxb.) Sweet	30	Present Communication	Andhra Pradesh, Bihar, Jharkhand, Maharashtra, Rajasthan, Tamil Nadu
32.	**Argyreia setosa var. minor* (C.B. Clarke) Staples & Traiperm	–	–	Karnataka, Maharashtra
33.	**Argyreia sikkimensis* (C.B.Clarke) Ooststr.	–	–	Arunachal Pradesh, Assam, Mizoram
34.	*Argyreia splendens* (Hornem.) Sweet	–	–	Assam, Nagaland, Meghalaya, Mizoram, Tripura
35.	**Argyreia srinivasanii* Subba Rao & Kumari	–	–	Andhra Pradesh
36.	*Argyreia thomsonii* (C.B.Clarke) Craib ex C.R.Babu	–	–	Uttarakhand, Uttar Pradesh, West Bengal
37.	*Argyreia venusta* Choisy	–	–	Assam, Meghalaya
38.	*Argyreia wallichii* Choisy	30	[1]	Assam, Manipur, Mizoram and Meghalaya
39..	*Argyreia wallichii var. coriacea* C.B.Clarke	–	–	Assam, West Bengal, Mizoram and Meghalaya
40.	*Argyreia zeylanica* (Gaertn.) Voigt	–	–	Tamil Nadu

*Represents Endemic taxa, – represents taxa where somatic counts are not known

In the genus *Argyreia* almost 80% of the species are yet to be cytologically investigated. Hence, the paper deals with the detailed karyomorphological studies of the three species of *Argyreia,* viz. *A. cuneata, A. osyrensis. A. setosa*. Of these three, cytotaxonomic details of *A. osyrensis* and *A. setosa* are reported for the first time.

Materials and methods

Three species of *Argyreia* were collected from their natural habitats from the Indian states of Maharashtra and Karnataka. Herbarium specimens of the species have been deposited in Shivaji University Herbarium (SUK). Seeds were nicked near the hilum, soaked in distilled water for 12 h and sown in soil for germination. Root tips ca. 1 cm in length were excised, pretreated for 3–4 h in *p*-Dichlorobenzene at 12–14° C, hydrolyzed in 1 N. HCl for 45 s and stained with 2% propionic-orcein. Suitable somatic plates were photographed with LEICA DM 2000 microscope fitted with camera at × 1000 magnification. The karyotypic analysis was done with 8–10 well separated mitotic plates. The chromosome nomenclature followed was that proposed by Levan et al. [4]. The relative variation in chromosome length was evaluated using CV_{CL} [8] parameter while the relative variation in centromeric position of chromosomes in complement was evaluated using CV_{CI} [8] parameter. Stebbins classification [16] and asymmetry index (AI) by Paszko [8] were employed to estimate overall asymmetries of the karyotypes.

Results

The three species studied had somatic chromosome count, $2n = 30$ chromosomes (Fig. 1b, d, f). The shortest chromosome pair observed was 2.43 µm from *A. cuneata*, while the longest chromosome pair of 4.81 µm incurred in *A. osyrensis*. *Argyreia osyrensis* had the highest Mean Chromosome Length (MCL) and highest Total Chromosome Length (TCL), 3.62 µm and 54.25 µm, respectively. *Argyreia setosa* had the lowest Mean Chromosome Length, 3.07 µm and lowest Total Chromosome Length (TCL), 46.04 µm. *Argyreia cuneata* had MCL and TCL values intermediate between the above two (Table 2).

The karyotypic formulae were deduced to be 8m + 7sm for *A. cuneata*, 5m + 10sm for *A. osyrensis* and 15m chromosome pairs for *A. setosa*. The karyotypes of *A. cuneata* and *A. osyrensis* belonged to the 3A category while that of *A. setosa* fitted with the 4A category of Stebbins [16] (Table 3). The karyotype asymmetry values of CV_{CL}, CV_{CI} and AI were highest for *A. osyrensis* and lowest for *A. setosa*. The values of CV_{CL}, CV_{CI} and AI for *A. cuneata* were occurred between the above two (Table 3).

Discussion

Somatic chromosome count, base number and ploidy level of species form the primary basis of cytotaxonomy [14]. In Convolvulaceae, $x = 5$ is believed to be a primary basic number. The $x = 15$, $x = 14$ is prevalent among the studied taxa. This may represent the secondarily derived basic numbers [6, 10]. The chromosomes in Convolvulaceae showed great variability in length. Sampathkumar [12] has recognized

Table 2 Karyotype data for *A. cuneata, A. osyrensis* and *A. setosa*

Taxon	Long arm ± SD (µm)	Short arm ± SD (µm)	TCL (µm)	MCL (µm)	r ± SD (µm)	R
A. cuneata	2.18 ± 0.33	1.32 ± 0.22	52.43	3.50	1.69 ± 0.15	1.83
A. osyrensis	2.28 ± 0.24	1.33 ± 0.34	54.25	3.62	1.84 ± 0.32	1.76
A. setosa	1.83 ± 0.25	1.24 ± 0.18	46.04	3.07	1.51 ± 0.11	1.67

SD standard deviation, *TCL* total chromosome length of the complement, *MCL* mean chromosome length of the complement, *r* mean arm ratio, *R* ratio between the largest and the smallest chromosomes of the complement

Table 3 Karyotype evaluation data using different methods to evaluate karyotype asymmetry

Taxon	2*n*	Karyotype formula	CV_{CL}	CV_{CI}	AI	Stebbins Type
A. cuneata	30	8m + 7sm	15.13	5.28	0.8	3A
A. osyrensis	30	5m + 10sm	15.45	10.75	1.66	3A
A. setosa	30	15m	13.44	4.12	0.55	4A

CV_{CL} the coefficient of variation in chromosome length, CV_{CI} The coefficient of variation of the centromeric index, *AI* asymmetry index

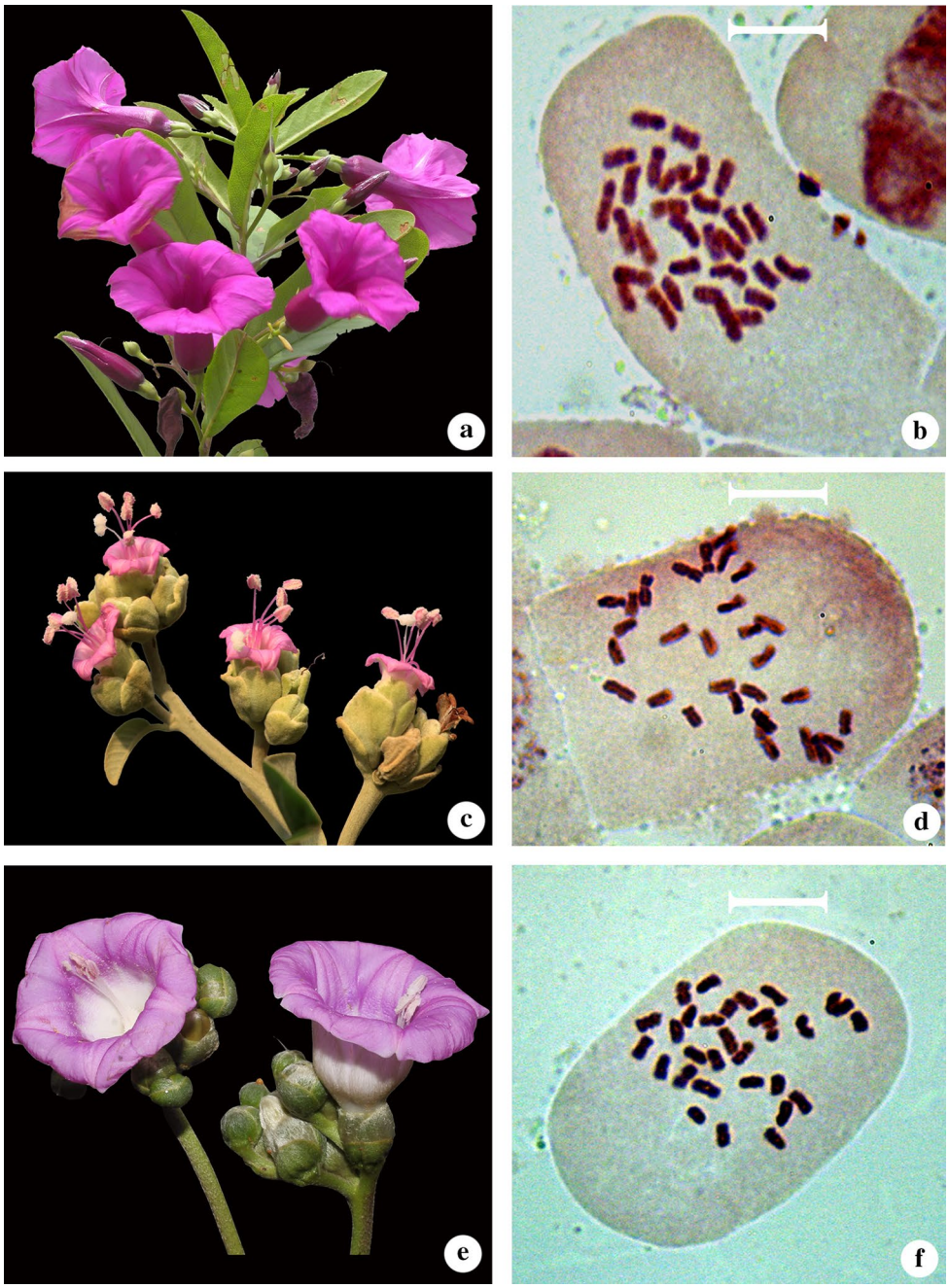

Fig. 1 *Argyreia cuneata*: **a** Flower, **b** Mitotic metaphase plate; *A. osyrensis*: **c** Flower with exserted stamens, **d** Mitotic metaphase plate; *A. setosa*: **e** Flower, **f** Mitotic metaphase plate. Scale bar = 10 µm

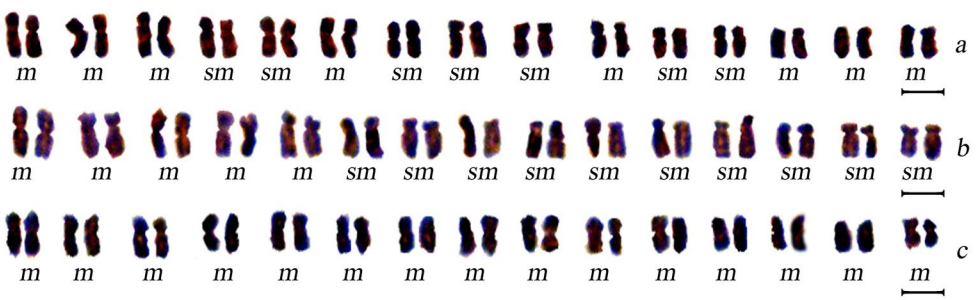

Fig. 2 Karyogram of *A. cuneata*; **b** Karyogram of *A. osyrensis*; **c** Karyogram of *A. setosa*. Scale bar = 10 µm

two categories of chromosomes: *Ipomoea* type (short) and *Argyreia* type (large). As compared to the other Convolvulaceae members, the genera *Argyreia, Rivea* and *Stictocardia* possess large chromosomes (*Argyreia* type) with centromere in the median and submedian region.

Two series of somatic chromosome counts, $2n = 2x = 30$ and $2n = 2x = 28$ chromosomes, exists in *Argyreia* and fall under two basic number series viz. $x = 15$ and $x = 14$ chromosomes respectively, with no reported polyploids. There is preponderance of base chromosome number $x = 15$ although either of them could have derived from the other through aneuploidy [10].

The somatic chromosome counts of *A. cuneata*, $2n = 30$ chromosomes found in this study is in congruence with the studies of Sampathkumar [12]. However, unlike Sampathkumar [12], who reported 7m + 8sm homologous pairs, we report 8 + 7sm homologous chromosome pairs in *A. cuneata*. Previous chromosome studies have shown the occurrence of satellite chromosomes but in our studies we have not found such satellite chromosomes.

According to Lorenzo and Halil [5] CV_{CI} index is a measure of chromosomal heterogeneity and not of intrachromosomal asymmetry although our results suggests it is useful to show intrachromosomal asymmetry. The CV_{CI} parameter measures relative variation in the centromeric position among chromosomes in complement. *Arygyeia osyrensis* exhibits the highest CV_{CI} percentage, reflected in the karyogram as an increase in the number of submetacentric chromosomes. The lowest CV_{CI} is in *A. setosa*, as is evident from its all-metacentric chromosomes (Table 3 and Fig. 2). The relative variation in chromosome length in a complement is evaluated by CV_{CL} parameter. The CV_{CL} value was high in *A. osyrensis* and was slightly variable in *A. cuneata* which is possibly due to their high total chromosomal length (Table 2, Table 3). The Asymmetry Index (AI) by Paszko [8], which combines two kinds of asymmetries, is proved beneficial in analyzing karyotype asymmetry in our study. The highest AI value in *A. osyrensis* represents a comparatively asymmetric karyotype while the lowest in *A. setosa* represents a symmetric karyotype (Table 3), which is also clear from their karyotypic formulae (Fig. 2).

According to previous studies, most of the karyotypes in the genus *Argyreia* have a preponderance of chromosomes with centromere in the median and sub median regions, suggesting moderately symmetrical nature of karyotypes. The karyotypes of *A. osyrensis* and *A. cuneata* fall in the 3A while that of *A. setosa* belong to the 4A category, which also implies a moderately symmetrical to symmetrical nature of karyotypes.

Argyreia, though cytologically poorly studied genus, is a good candidate for cytogenetic studies for establishment of infrageneric classification and better understanding of its taxonomy.

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