Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: R.N. Chougule, P.V. Deshmukh, P.E. Shelke, V.J. Patil, M.M. Lekhak (2023). Cytogenetical studies of some Convolvulaceae members from the Western Ghats, India reveal uniformity in karyotypes. *Caryologia* 76(2): 59-65. doi: 10.36253/caryologia-2304

Received: September 8, 2023

Accepted: October 29, 2023

Published: December 31, 2023

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Cytogenetical studies of some Convolvulaceae members from the Western Ghats, India reveal uniformity in karyotypes

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Abstract. Karyotypes of six *Ipomoea* and one *Merremia* species were studied. Chromosome counts of $2n = 30$ for *I. corymbosa, I. kotschyana, I. marginata* f. *candida* and *M. rhyncorhiza)* and $2n = 60$ for *I. ochracea* were observed for the first time. Meiotic course of five *Ipomoea* species was also examined for the first time and two counts, i.e. $n = 15$ and $n = 30$ were recorded. Meiosis was found normal. Karyotypes of the studied species exhibited uniformity. All the species had chromosomes with median region centromeres and karyotypes were symmetrical (Stebbins' 4A category). *I. marginata* f. *candida* had the shortest chromosomes with mean length of 0.99 ± 0.02 µm. *I. ochracea* and *I. corymbosa* had the longest chromosomes with mean length of 1.22 ± 0.05 µm and 1.22 ± 0.01 µm, respectively. As chromosomes were small and exhibited uniform morphology, fluorescent banding or fluorescent in-situ hybridization can be useful to differentiate the karyotypes and understand species interrelationships.

Keywords: chromosomes, morning glory, karyotype symmetry, Western Ghats.

INTRODUCTION

Convolvulaceae, called as *bindweeds* or *morning glories* are a family of about 58 genera and more than 1977 species worldwide (POWO 2023). In India, there are about 24 genera that include about 164 species of which 27 are endemic (Dash and Mao 2020; Singh et al. 2015). These species are mostly herbaceous to woody vines, rarely herbs, shrubs, or trees. India is home to a rich diversity of Convolvulaceae due to the presence of diverse habitats and exposure to introduced species. *Ipomoea* L. is a large genus that comprises more than 800 species distributed over tropical and subtropical regions of the world (Wood et al. 2020). In India, the genus is represented by (64 taxa) 55 species and 9 infraspecific taxa, of which only three species, *I. clarkei* Hook.f, *I. laxiflora* H.J.Chowdhery & Debta and *I. salsettensis* Santapau & Patel are endemic (Dash and Mao 2020; Singh et al. 2015). Some taxa such as *I. cairica* var. *semine-glabro* (Blatt. & Hallb.) Bhandari, *I. marginata* f. *candida* (Naik & Zate) Das Das & Lakshmin., *I. deccana* var. *lobata* (C.B.Clarke)

S.C.Johri, *I. nil* var. *himalaica* (C.B.Clarke) S.C.Johri, *I. obscura* f. *concolor* Naik & Zate, *I. pes-caprae* var. *perunkulamensis* P.Umam. & P.Daniel have also been reported as endemic to India (Kattee 2019). The Western Ghats consists of 42 taxa (37 species, 03 subspecies and 02 varieties) (Lekhak et al. 2018). *Merremia* Dennst. ex Endl., on the other hand includes 49 species distributed over tropical and subtropical regions of the world (POWO 2023). In India, *Merremia* comprises 17 species, of which two species, *M. rajasthanensis* Bhandari and *M. rhyncorhiza* (Dalzell) Hallier f. are endemic to the country (Dash and Mao 2020; Singh et al. 2015).

Convolvulaceae members are economically very important. For instance, *I. batatas* (L.) Lam., popularly known as sweet potato, is a rich source of energy for humans as well as animals. The plant parts of *I. pes-caprae* (L.) R.Br. have been traditionally used to treat gastrointestinal-related disorders and symptoms, such as dysentery, ulcer, abdominal pain, cramps and stomach aches (Emendörfer et al. 2005; Pereda-Miranda et al. 2005). Many members such as *Convolvulus* L., *Ipomoea*, *Stictocardia* Hallier f., etc. are used as ornamentals. Moreover, the members of the family serve as an important source of ergoline alkaloids (Fig. 1) that have been used to treat nervous system disorders like convulsions, epilepsy, migraine, Parkinson's disease or are used in childbirth and weaning (Groeger and Floss 1998; Mutschler et al. 2001; Schardl et al. 2006; Chen et al. 2018).

On account of its tremendous utility in medicinal and ornamental fields, the family is cytogenetically well explored. In India, somatic chromosomes of these members have been the focal point for the majority of studies (Vij et al. 1974, 1977; Bir and Sidhu 1975; Bir et al. 1978; Roy 1979; Sampathkumar 1979; Rao and Mwasumbi 1981; Sinha and Sharma 1992; Rane et al. 2012; Lekhak et al. 2018; Lawand et al. 2019; Ramanpreet and Gupta 2018). Most of these studies recorded chromosome counts for species of *Ipomoea*, *Argyreia* Lour., *Merremia* and *Operculina* Silva Manso. The most common diploid chromosome number reported in these genera is $2n = 30$. Although, $2n$ with 18, 22, 28, 32, 38, 58 and 60 chromosomes have also been reported in some species (Yeh and Tsai 1995; Lekhak et al. 2018; Lawand et al. 2019). Instances of polyploidy are usually rare. Nevertheless, polyploidy has been reported in *Ipomoea batatas* which is a hexaploid ($2n = 6x = 90$) (Sinha and Sharma 1992; Vij et al. 1977).

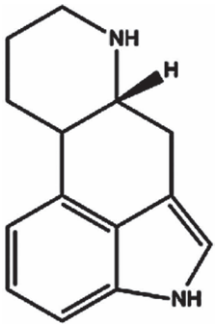

Figure 1. Structure of Ergoline alkaloid.

The present investigation aims at generating new cytogenetical data for the flowering plants (wild or introduced) of the Western Ghats, India. Since Convolvulaceae members are of horticultural and medicinal importance, chromosomal information would be useful in understanding their genetic potential, phylogenetic relationships and breeding strategies. Accordingly, here we provide comparative karyotypes of seven species. Meiotic chromosomes were studied for five *Ipomoea* species. Karyological analysis of taxa was based on karyological parameters such as diploid chromosome number ($2n$), mean chromosome length (MCL), total haploid chromosome length (THL), mean centromeric asymmetry (M_{CA}) and coefficient of variation of chromosome length (CV_{CL}).

MATERIAL AND METHODS

The plant materials for the present study were collected from different localities in Gujarat and Maharashtra state. Plants were cultivated in Lead Botanical Garden, Department of Botany, Shivaji University, Kolhapur and the voucher specimens deposited in the Herbarium of Shivaji University, Kolhapur (SUK) (Table 1). For mitotic preparations, seeds were nicked near the hilum with the help of a sharp razor, and then placed in the petri dish on a wet blotting paper. Well-grown root tips (1.5-3 cm long) were harvested from the germinating seeds and pre-treated with a saturated solution of *para*-Dicholorobenzene (*p*DB) for 4-5 h at 9±3°C. Further, the root tips were hydrolysed in 1N HCl and squashed in 2% propionic orcein. For meiotic studies, appropriately sized flower buds were fixed in Carnoy's solution and smears of anthers from floral buds were stained using 2% propionic orcein. Suitable somatic and meiotic plates from freshly prepared slides were photographed with Leica DM 750 microscope with attached camera at 1000X magnification. Five cells with well-spread metaphase chromosomes were selected for karyotype analysis. Nomenclature of chromosomes follows Levan et al. (1964). Karyotype asymmetry was ascertained using CV_{CL} (coefficient of variation of chromosome length)

Table 1. Collection localities and voucher details of studied species.

Sr. No.	Taxa	Collection locality	Voucher specimen
1.	*Ipomoea corymbosa* (L.) Roth	Shivaji University Campus, Kolhapur, Maharashtra	RNC 127
2.	*I. horsfalliae* Hook.	Uajalaiwadi, Kolhapur district, Maharashtra	RNC 121
3.	*I. kotschyana* Hochst. ex Choisy	Dinodhar hill, Nakhatrana taluka, Kutch district, Gujarat	RNC 206
4.	**I. laxiflora* H.J.Chowdhery & Debta	Gujarat	RNC 277
5.	**I. marginata* f. *candida* (Naik & Zate) Das Das & Lakshmin.	Shivaji University Campus, Kolhapur, Maharashtra	RNC 207
6.	*I. ochracea* (Lindl.) Sweet	Shivaji University Campus, Kolhapur, Maharashtra	RNC 120
7.	**I. salsettensis* Santapau & Patel	Rajapur, Ratnagiri district, Maharashtra	RNC 278
8.	**Merremia rhyncorhiza* (Dalzell) Hallier f.	Chaukul, Sawantwadi taluka, Sindhudurg district, Maharashtra	RNC 217

*Indicates endemic species.

Table 2. Comparative karyotypes.

Sr. No.	Taxa	2*n*	Range of chromosome length ± SE (μm)	Arm ratio (r) ± SE	THL (μm)	MCL ± SE (μm)	M_{CA}	CV_{CL}	R	St	Haploid karyotype formulae
1.	*I. corymbosa*	30	1.56 ± 0.02 - 0.90 ± 0.03	1.16 ± 0.01	18.35	1.22 ± 0.05	7.42	14.70	1.74	4A	15m
2.	*I. kotschyana*	30	1.21 ± 0.01 - 0.74 ± 0.01	1.22 ± 0.01	14.65	0.98 ± 0.03	14.65	13.16	1.65	4A	15m
3.	*I. laxiflora*	30	1.35 ± 0.04 - 0.86 ± 0.02	1.15 ± 0.04	15.76	1.05 ± 0.02	7.05	12.66	1.58	4A	15m
4.	*I. marginata* f. *candida*	30	1.20 ± 0.01 - 0.74 ± 0.02	1.22 ± 0.02	14.80	0.99 ± 0.02	8.84	12.28	1.65	4A	15m
5.	*I. ochracea*	60	1.57 ± 0.02 - 0.94 ± 0.01	1.21 ± 0.03	36.61	1.22 ± 0.01	9.26	12.85	1.66	4A	30m
6.	*I. salsettensis*	30	1.51 ± 0.07 - 0.95 ± 0.04	1.16 ± 0.04	17.85	1.19 ± 0.04	7.39	12.68	1.59	4A	15m + (0–4B)
7.	*M. rhyncorhiza*	30	1.38 ± 0.04 - 0.85 ± 0.02	1.15 ± 0.01	16.31	1.09 ± 0.04	6.85	12.83	1.61	4A	15m

THL = Total haploid length, MCL = Mean chromosome length, M_{CA} = Mean centromeric asymmetry, CV_{CL} = Coefficient of variation of chromosome length, R = ratio of the longest to shortest chromosome of a complement, St = karyotype asymmetry.

and M_{CA} (mean centromeric asymmetry) as suggested by Peruzzi and Eroğlu (2013).

RESULTS

In the present investigation mitotic metaphase chromosomes of seven species were studied. Meiotic study was performed on five species. *Ipomoea ochracea* (Lindl.) Sweet exhibited 2*n* = 60 chromosomes while the rest of the species had 2*n* = 30 chromosomes. Comparative karyotypes of all the species investigated are summarized in Table 2. Fig. 2 illustrates the mitotic metaphases while Fig. 3 depicts ideogram. Chromosomes were with median region centromeres, and hence the karyotype formula 15m (*I. corymbosa, I. kotshyana, I. laxiflora, I. marginata* f. *candida* and *M. rhyncorhiza*) or 30m (*I. ochracea*). Four B-chromosomes were observed in *I. salsettensis* (Fig. 2f) and the karyotype formula was 15m+4B. The highest mean chromosome length (MCL) (1.22 ± 0.05 and 1.22 ± 0.01 μm) was recorded in the case of *I. corymbosa* and *I. ochracea* whereas the lowest (0.98 ± 0.03 μm) in *I. kotschyana* (Table 2). Total haploid chromosome length (THL) ranged from 14.65 μm (*I. kotschyana*) to 36.61 μm (*I. ochracea*). *I. corymbosa* showed maximum value (1.74) for R (ratio of largest and the smallest chromosome of the complement) while the minimum (1.58) was recorded for *I. laxiflora*. M_{CA} was found to be the lowest (6.85) for *M. rhyncorhiza* and the highest (14.65) for *I. kotshyana*. The lowest CV_{CL} was recorded for *I. marginata* f. *candida* (12.28) and the highest for *I. corymbosa* (14.70) (Table 2).

Meiotic studies were carried out in five species (*I. horsfalliae*, *I. laxiflora*, *I. marginata* f. *candida*, *I. ochracea*, *I. salsettensis*). Meiosis was found to be normal. Pollen mother cells (PMCs) of *I. ochracea* showed 30 bivalents (*n* = 30) at diakinesis (Fig. 2k) whereas rest of the species (*I. horsfalliae*, *I. laxiflora*, *I. marginata* f. *candida* and *I. salsettensis*) showed 15 bivalents (*n* = 15) (Fig. 2h, i, j, l).

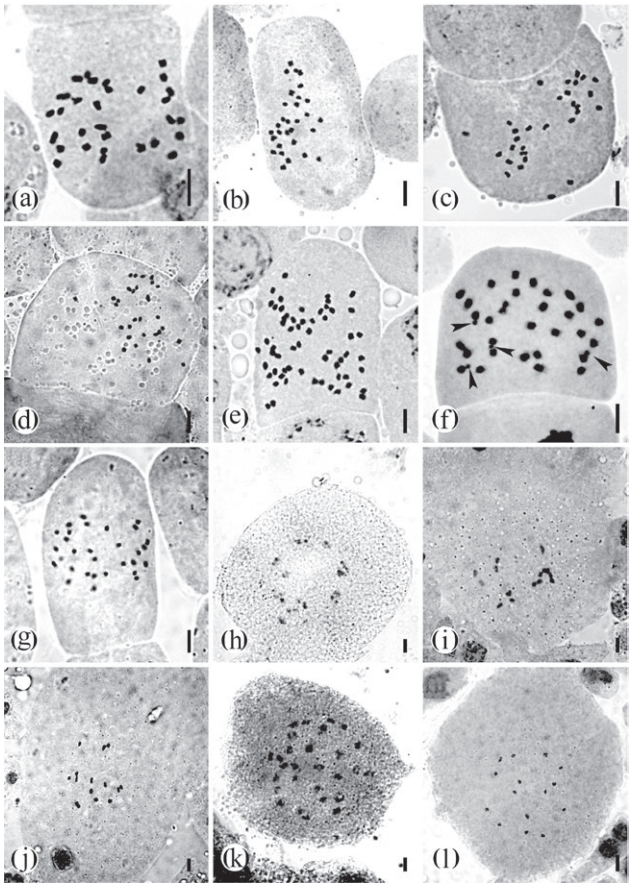

Figure 2. Mitotic metaphase and meiotic chromosomes: (a) *Ipomoea corymbosa* (2*n* = 30); (b) *I. kotschyana* (2*n* = 30); (c) *I. laxiflora* (2*n* = 30); (d) *I. marginata* f. *candida* (2*n* = 30); (e) *I. ochracea* (2*n* = 60); (f) *I. salsettensis* (2*n* = 30+4B) Arrowheads show B-chromosomes; (g) *Merremia rhyncorhiza* (2*n* = 30). (h-l) PMCs at diakinesis: (h) *I. horsefalliae* (*n* = 15); (i) *I. laxiflora* (*n* =15); (j) *I. marginata* f. *candida* (*n* = 15); (k) *I. ochracea* (*n* = 30); (l) *I. salsettensis* (*n* = 15). Scale bars = 5 µm.

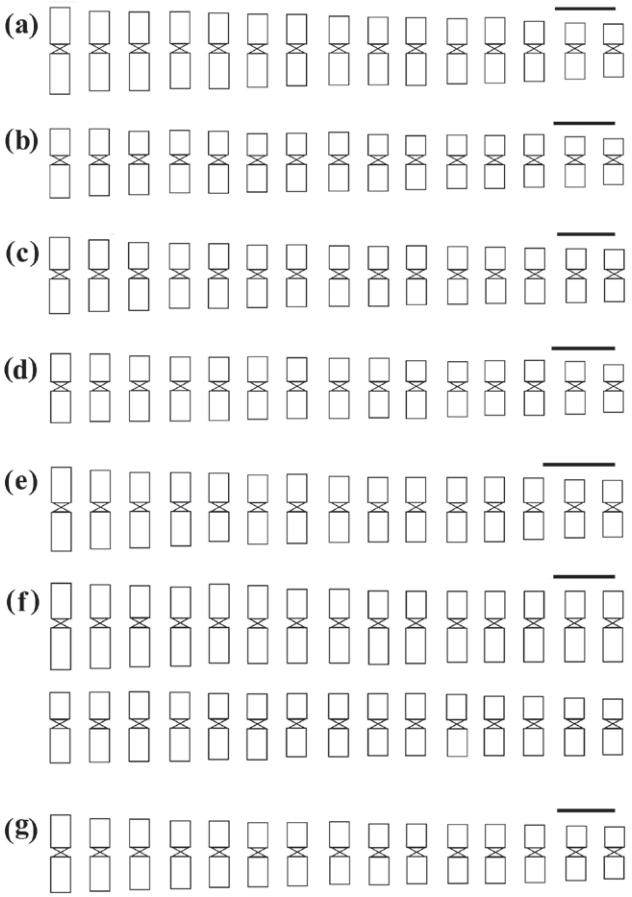

Figure 3. Ideograms of *Ipomoea* and *Merremia* species: (a) *I. corymbosa*; (b) *I. kotschyana*; (c) *I. laxiflora*; (d) *I. marginata* f. *candida*; (e) *I. salsettensis*; (f) *I. ochracea*; (g) *M. rhyncorhiza.* Scale bars = 1 µm.

DISCUSSION

According to Löve and Löve (in Sinha and Sharma 1992) $x = 5$ is the primary basic chromosome number for the family Convolvulaceae while $x = 14$ and $x = 15$ are secondarily derived basic numbers. Darlington and Wylie (1955) and Vij et al. (1977) considered $x = 14$ and $x = 15$ as the basic chromosome numbers for the genus *Ipomoea* and *Merremia*. Most of the Indian species exhibit a diploid chromosome number of $2n = 30$. In the present studies we observed $2n = 30$ chromosomes in six species whereas $2n = 60$ was found in *I. ochracea*. Earlier, the count of $2n = 60$ has been observed as an instance of tetraploidy in *I. wightii* (Wall.) Choisy, *I. plebeia* R.Br., *I. lonchophylla* J.M.Black, *I. racemigera* F.Muell. & Tate, *I. ramonii* Choisy, *I. tiliacea* (Willd.) Choisy and *I. arborescens* (Humb. & Bonpl. ex Willd.) G.Don (Jones 1964; Yen et al. 1992; Kattee 2019) whereas, *I. batatas* shows hexaploidy, i.e. $2n = 90$ chromosomes (Sinha and Sharma 1992; Pitrez et al. 2008). Some species such as *I. staphylina* Roem. & Schult., *I. purpurea* (L.) Roth possess $2n = 32$ chromosomes (Roy 1979; Sampathkumar 1979; Dhar 2015) whereas *I. coptica* Verdc., *I. diversifolia* R.Br., *I. pes-tigridis* L. show $2n = 28$ chromosomes (Sampathkumar 1979; Lekhak et al. 2018; Bir and Sidhu 1975). *I. triloba* L. is reported to have $2n = 38$ chromosomes (Wang et al. 1998). These chromosome numbers not confirming to base numbers $x = 14$ and $x = 15$ can be attributed to polyploidy, aneuploidy or dysploidy or to the occurrence of polysomaty (Vij et al. 1977; Sampathkumar 1979; Lekhak et al. 2018).

Sampathkumar (1979) studied the karyomorphology of eighteen *Ipomoea* species whereas Rane et al. (2012) studied ten species. In both the studies chromosomes

with median and submedian centromeres were observed. Nakajima (1963) also reported median, submedian and terminal centromere in *I. lacunosa* L. and *I. violacea* L. and hence, the karyotype was considered asymmetrical. In the present study we observed all the chromosomes with median region centromere and karyotype was highly symmetrical (Table 2). Similar observations have been made for *I. clarkei* and *I. diversifolia* (Lekhak et al. 2018) and in some NE Brazilian *Ipomoea* species (Pitrez et al. 2008). Kattee (2019) reported two counts, i.e. $2n$ = 30 (*I. laxiflora*, *I. salsettensis* and *I. tenuipes* Verdc.) and $2n$ = 60 (*I. wightii*). She observed chromosomes with median region centromere. In the present study, the chromosome number for *I. laxiflora* and *I. salsettensis* have been confirmed, although four B-chromosomes were observed in *I. salsettensis* for the first time (Fig. 2f). B-chromosomes (0-1) have also been reported in other *Ipomoea* species such as *I. mutabilis* Lindl. and *I. palmata* Forssk. (white flowered type) by Vij et al. (1977) whereas Yen and Tsai (1995) observed B-chromosomes (0-3) in some *Ipomoea* species from Taiwan. Sampathkumar (1979) recorded the presence of a satellite chromosome pair and secondarily constricted chromosomes in *Ipomoea* species. Similarly, Pitrez et al. (2008) observed satellite chromosomes in some *Ipomoea* species from NE Brazil inselbergs. In the present investigation, we could not find satellite chromosomes or secondarily constricted chromosomes.

Chromosome length ranged from 2.13 to 4.79 µm in *I. carnea* Jacq. and 1.25 to 2.67 µm in *I. aquatica* Forssk. (Rane et al. 2012). Lekhak et al. (2018) reported the shortest chromosome in *I. diversifolia* (1.62 µm) and the longest in *I. clarkei* (2.15 µm). In the present investigation, the longest chromosomes were observed in *I. ochracea* (0.94 to 1.57 µm) whereas the smallest (0.74 to 1.20 µm) in *I. marginata* f. *candida*. All the studied species fell under Stebbins's karyotype asymmetry class 4A. M_{CA} value was maximum in *I. kotschyana* (14.65) which indicated greater differences in the centromeric position across the chromosome complement whereas the highest CV_{CL} in *I. corymbosa* (14.70 µm) was on account of higher heterogeneity in the length of the chromosome complement.

Amongst the Indian *Merremia* species, cytogenetical data are available for 60% species (after Rice et al., 2015). Lewis et al. (1967) recorded $2n$ = 28 chromosomes for *M. aegyptia* (L.) Urb. whereas $2n$ = 30 chromosomes were reported by Jones (1968) and Pitrez et al. (2008). Vij et al. (1977) observed $2n$ = 30 chromosomes in *M. dissecta* (Jacq.) Hallier f. and *M. aegyptia*. Meiosis revealed n = 15 bivalents for both *M. dissecta* and *M. aegyptia*. Aberrant meiosis was reported in *M. aegyptia*. The presence of $2n$ = 28 and 30 chromosomes need to be further investigated. Secondly, this could also be possible due to the existence of two cytotypes in *M. aegyptia*. Ramanpreet and Gupta (2018) recorded n = 7 bivalents in *M. umbellata* (L.) Hallier f. Sampathkumar (1979) reported $2n$ = 32 and $2n$ =30 chromosomes in *M. dissecta* and *M. hederacea* (Burm.f.) Hallier f., respectively with median and submedian centromeres. R value was 2.5 and 3.3 and the chromosome length ranged from 1.2 µm to 3.0 µm and 1.0 µm to 3.3 µm for *M. dissecta* and *M. hederacea*, respectively. We observed $2n$ = 30 chromosomes in *M. rhyncorhiza*. Accordingly, the R value, i.e. 1.61 was smaller than *M. dissecta* and *M. hederacea*. The chromosomes were smaller in size (0.85 µm to 1.38 µm) and had median region centromeres and symmetrical karyotype. Pitrez et al. (2008) also reported symmetrical karyotype in *M. aegyptia* but the chromosomes were with metacentric and submetacentric region centromere with terminal secondary constriction on one of submetacentric pairs. Sampathkumar (1979) also found satellite chromosomes and secondary constrictions in both species *M. dissecta* and *M. hederacea*. We could not observe any satellite chromosomes and secondary constrictions in *M. rhyncorhiza*.

Recently, Ramanpreet and Gupta (2018) carried out meiotic studies on 19 species of Convolvulaceae from Indian hot desert Rajasthan. They studied nine *Ipomoea* species and reported normal meiosis and high pollen fertility in all studied *Ipomoea* species. For *I. cordatotriloba* Dennst., *I. triloba* and *I. sagittifolia* Burm.f. a meiotic count of n = 15 bivalents was recorded for the first time (Ramanpreet and Gupta 2018). They found n = 15 bivalents for eight *Ipomoea* species and n = 14 bivalents for *I. pes-tigridis*. This study also confirmed earlier reports on chromosome numbers of *Ipomoea*. In present investigation, meiotic counts of n = 15 in *I. horsfalliae, I. laxiflora*, *I. marginata* f. *candida* and *I. salsettensis* and n = 30 in *I. ochracea* were made for the first time. Meiotic course was normal. Lekhak et al. (2018) also found normal meiosis in *I. clarkei* and *I. diversifolia*. Vij et al. (1977) also studied meiosis in *Ipomoea* and some allied genera. They studied meiosis in 22 *Ipomoea* species and found normal bivalent formation. Most of the *Ipomoea* species exhibited n = 15 bivalents. *I. coccinea* L. and *I. batatas* were reported to have the counts of n = 14 and n = 45, respectively. Irregular anaphases were also observed some species. One B-chromosome was observed in *I. mutabilis* and *I. palmata* (white flowered type). We did not find B-chromosomes in the meiotic phases. Chromosome data are now lacking only in fourteen *Ipomoea* and six *Merremia* species in India (Table 3).

Table 3. Indian species of *Ipomoea* and *Merremia* awaiting karyological investigation and their geographical distribution. #Indicates introduced species and *endemic species.

Sr. No.	Taxa	Geographical distribution
1.	*Ipomoea acanthocarpa* (Choisy) Hochst. ex Schweinf. & Asch.	Gujarat
2.	*I. aitonii* Lindl.	Telangana
3.	*I. barlerioides* (Choisy) Benth. ex C.B.Clarke	Andhra Pradesh, Bihar, Daman & Diu, Goa, Himachal Pradesh, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Odisha, Punjab, Sikkim, Tamil Nadu, Uttar Pradesh, West Bengal
4.	*I. cairica* var. *semine-glabro* (Blatt. & Hallb.) Bhandari	Rajasthan
5.	*I. marginata* f. *marginata*	Throughout India
6.	*I. nil* var. *himalaica* (C.B.Clarke) S.C. Johri	Sikkim, Jammu and Kashmir to Rajasthan
7.	*I. obscura* f. *concolor* Naik & Zate	Maharashtra
8.	*I. pes-caprae* var. *perunkulamensis* P.Umam & P. Daniel	Tamil Nadu
9.	#*I. heptaphylla* Sweet	Native range Tropical & Subtropical America
10.	*I. rubens* Choisy	Assam, Maharashtra, West Bengal
11.	*I. rumicifolia* Choisy	Rajasthan, Gujarat, Kerala, Tamil Nadu
12.	*I. tuberculata* Ker Gawl.	Gujarat, Himachal Pradesh, Kerala, Maharashtra, Tamil Nadu, Uttar Pradesh, West Bengal
13.	*I. vagans* Baker	Gujarat
14.	*I. velutina* R.Br.	West Bengal
15.	*M. cissoides* (Lam.) Hallier f.	Kerala
16.	*M. kentrocaulos* (C.B.Clarke) Hallier f.	Andhra Pradesh, Kerala, Tamil Nadu
17.	*M. mammosa* (Lour.) Hallier f.	Andaman & Nicobar Islands, Arunachal Pradesh, Assam
18.	*M. quinata* (R.Br.) Ooststr.	Bihar, Odisha, Tamil Nadu
19.	*M. rajasthanensis* Bhandari	Rajasthan
20.	*M. sibirica* (L.) Hallier f.	Himachal Pradesh, Uttar Pradesh

CONCLUSION

Comprehensive data on cytogenetics of *Ipomoea* and *Merremia* are important to understand the chromosomal evolution and harness the economic potential. As molecular phylogeny for the Indian taxa is not available, karyological data, particularly chromosome number and information from fluorescent banding or fluorescent in-situ hybridization can help to reveal species interrelationships. Based on the data of chromosome number for *Ipomoea* and *Merremia* presented here and previous reports it is clear that there are two basic chromosome numbers, i.e. $x = 14$ and $x = 15$. Nevertheless, more information on the hitherto studied taxa and confirmation of chromosome number in taxa where the count does not confirm to these numbers is needed to understand mechanisms underlying evolution in these genera.

ACKNOWLEDGEMENTS

Authors are grateful to the Head, Department of Botany, Shivaji University, Kolhapur (SUK) for providing research facilities. RNC thanks DBT for financial support under 'Network Programme for Enrichment and Update of Plant Chromosome Database for Spermatophytes and Archegoniate Phase - II' vide sanction letter BT/PR34491/NDB/39/678/2020 dated 11 November 2021.

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