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ORIGINAL ARTICLE

Chromosomal variation in some members of Malvales Juss. from Rajasthan, India

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Abstract The present paper deals with the meiotic study of 15 species belonging to 7 genera of 3 families of order Malvales i.e. Malvaceae, Sterculiaceae and Tiliaceae from different geographical areas of Rajasthan. Out of 15 species, intraspecific cytotypes at tetraploid and diploid level of *Triumfetta rhomboidea* has been reported for the first time. The chromosome numbers of 6 species i.e. *Abutilon pannosum*, *Sida ovata*, *Sida rhombifolia*, *T. rhomboidea* are reported for the first time from India. Whereas, chromosome studies on *Melhania magnifolia* have been worked out for the first time, also the occurrence of new cytotypes of *Abutilon ramosum* (2n = 42) are the first record in global context. Meiotic course is found to be abnormal in case of *T. rhomboidea, A. pannosum* and *A. ramosum* with the presence of laggards, chromatin bridges, unoriented bivalents and cytomixis, leading to the reduced pollen fertility and heterogenous sized pollen grains.

Keywords Malvales · Chromosome variation · Cytotype · Base numbers · Rajasthan

Introduction

The families Malvaceae, Sterculiaceae and Tiliaceae have been classified under the order Malvales [4]. The family Malvaceae Juss. comprises of 245 genera and 4300 species [3]. In India, the family is represented by 22 genera and 93 species [30]. A perusal of literature shows that cytological investigations in the family have been carried out by various workers from outside India [8, 15, 28].

The family Tiliaceae comprises of 450 species belonging to 50 genera [35], including 8 genera and 53 species from India [29]. According to the literature, cytological investigations, in the family have been carried out [7, 27] from outside India. From Indian subcontinent, 70 species and 13 genera of this family are chromosomally studied [17, 22, 33]. While the family Sterculiaceae consists of 68 genera and 1100 species worldwide out of which 19 genera and 68 species have been found from India.

Cytological studies on different families of order Malvales have been done by various workers from India and abroad [6, 10, 11, 15, 18, 27]. Inspite of various contributions on the cytological aspects of different families belonging to Malvales throughout India, some areas are cytologically still unexplored. So the present research has been undertaken to know the cytological behaviour of various taxa belonging to Malvales from Rajasthan.

Materials and methods

For meiotic studies, flower buds were collected from different localities of selected areas of Rajasthan (Table 1). Smears of appropriate sized flower buds were made after fixing in Carnoy's fixative (6:3:1 volume ratio of Absolute ethanol:Chloroform:Acetic acid), using standard acetocarmine technique. Pollen viability was estimated by mounting mature pollen grains in glycerol–acetocarmine (1:1) mixture. Well-filled pollen grains with stained nuclei

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Table 1 Enumeration of the studied taxa, their collection site and previous report

S. no.	Taxon	Accession number (PUN)	Pollen viability (%)	Locality with altitude (m)	Previous chromosome reports
FAMILY: MALVACEAE					
1.	*Abelmoschus moschatus* Medik.	59671	100	Near Fatehsagar lake, Udaipur (580 m)	2n = 72
2.	*Abutilon pannosum* (G. Forst.) Schltdl.	59754	68.4	Sri Ganganagar (164 m)	2n = 42
3.	*Abutilon persicum* (Burm. f.) Merr.	59714	98	Jhalawar (320 m)	2n = 32, 42
4.	*Abutilon ramosum* (Cav.) Guill. & Perr.	59672	78	Gulab Bagh, Udaipur (598)	2n = 16
5.	*Hibiscus lobatus* (Murray) Kuntze	59680	99	Saheliyon Ki Bari, Udaipur (598 m)	2n = 34, 36, 72
6.	*Sida alba* Cav.	59746	98.02	BSI, Jodhpur (403 m)	2n = 14
7.	*Sida cordata* (Burm. f.) Borss. Waalk.	59743	100	Ranakpur, Pali (484 m)	2n = 16, 28, 34
8.	*Sida ovata* Forssk.	59730	99	Saheliyon ki Bari, Udaipur (598 m)	2n = 28, 56
9.	*Sida rhombifolia* L.	59679	100	Monsoon Palace, Udaipur (910 m)	2n = 14 + 1B, 16, 18, 28 + 0-7B, 36
FAMILY: STERCULIACEAE Vent.					
10.	*Melhania magnifolia* Blatt. & Hallb.	59670	100	On the way to Monsoon Palace, Udaipur (910 m)	No previous report
FAMILY: TILIACEAE Juss.					
11.	*Corchorus aestuans* L.	59744	82	Bheemsagar Dam, Jhalawar (322 m)	2n = 14
12.	*Corchorus olitorius* L.	59739	100	Saheliyon ki Bari, Udaipur (598 m)	2n = 14, 28
13.	*Triumfetta pentandra* A. Rich.	59687	100	Gulab bagh, Udaipur (591)	2n = 16, 32
14.	*Triumfetta rhombidea* Jacq.				
	P-1	58591	74	Jhalawar (469 m)	2n = 32, 48
	P-2	59697	92	Monsoon Palace, Udaipur (910 m)	
15.	*Triumfetta rotundifolia* Lam.	59732	96	Monsoon Palace, Udaipur (910 m)	2n = 32

Table 2 Data on cytomixis and meiotic course in the studied accessions of Malvales from different areas of Rajasthan

Accession no.	Cytomixis		Meiotic course		
	PMCs involved (%)	No. of PMCs involved	PMCs with Interbivalent connections	PMCs with bridge (at A-I/II, T-I/II) (%)	PMCs with laggards (at A-I/II, T-I/II) (%)
59754	–	–	–	8.5 ± 0.42 (8/94)	11.95 ± 0.59 (11/92)
59672	–	–	8.5 ± 0.42 (9/105)	–	–
59744	16.8 ± 0.8 (21/125)	3–5	7.9 ± 0.395 (7/88)	–	–
58591	18.44 ± 0.92 (19/103)	2–4	12.12 ± 0.606 (12/99)	–	15.23 ± 0.76 (16/105)

were taken as apparently fertile, while shrivelled and unstained pollen grains were counted as sterile. Photomicrographs of pollen mother cells and pollen grains were made from freshly prepared slides using Nikon 80i eclipse Digital Imaging System. Voucher specimens are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN) (Tables 1, 2, 3).

Results

Chromosome count

Presently, 16 populations of 15 species belonging to order Malvales have been worked out from different districts of Rajasthan having altitudinal range of 164–910 mts.

Table 3 Data on abnormal microsporogenesis in different accessions of order Malvales from different areas of Rajasthan

Accession no.	Monad		Dyads		Triads		Tetrads	
	WMN (%)	WM (%)	WMN (%)	WM (%)	WMN (%)	WM (%)	WMN (%)	WM (%)
59754	–	–	–	–	5.4 (5/91)	–	93.3 (98/105)	2.8 (3/105)
59744	2.6 (2/75)	–	–	–	3.15 (3/95)	–	97.3 (109/112)	2.6 (3/112)
58591	3.3 (3/89)	–	2.19 (2/91)	–	7.9 (7/88)	–	96.5 (111/115)	2.02 (2/99)

WMN without micronuclei, *WM* with micronuclei

FAMILY: MALVACEAE Juss.

Abelmoschus moschatus Medik.: Meiotic study of presently worked out accession depicts the presence of n = 18 (Fig. 1a) which is or possibly the first euploid cytotype at world level. Earlier, the species is known to have 2n = 72 from India and abroad [9].

Abutilon pannosum (G. Forst.) Schltdl.: Present chromosome count of the species, n = 21 (Fig. 1b) is most probably the first hexaploid cytotype worked out from India. Earlier the species is known to have 2n = 42 from outside of India [1, 19].

Abutilon persicum (Burm. f.) Merr.: Present chromosome number of n = 21 (Fig. 1c) corroborates with previous reports from India and outside of India.

Abutilon ramosum Guill. & Perr.: Presently worked out species reveals the chromosome count of 2n = 42 (Fig. 1d) which is or possibly the new hexaploid cytotype for the species (Fig. 1d) from world level. Earlier the species is known to have 2n = 16 from India [16].

Hibiscus lobatus (Murray) Kuntze: Present chromosome count of the species reveals the presence of n = 36 (Fig. 1e) which is in accordance with the earlier report [24] from South India. In spite of 2n = 72, 2n = 34 and 36 have also been reported from India and outside India.

Sida alba Cav.: Chromosomal study of the species reveals the presence of n = 7 (Fig. 1f) which is in conformity with previous reports from India and outside India.

Sida cordata (Burm. f.) Borss. Waalk.: Presently, the worked out species reveals the presence of n = 16 (Fig. 1g) which is in accordance with the previous reports from India and outside India.

Sida ovata Forssk.: The present chromosome count depicts the presence of n = 14 (Fig. 1h) which corroborates the previous reports from outside India but is cytologically worked out for the first time from India.

Sida rhombifolia L.: The species is worked out for the first time from India with a diploid cytotype of n = 8 (Fig. 1i). The other chromosome counts like 2n = 14, 18, 28 and 36 have also been reported from India and outside India.

FAMILY: STERCULIACEAE Vent.

Melhania magnifolia Blatt. & Hallb.: Meiotic study of the species depicts the presence of n = 30 (Fig. 1j) and the species is cytologically worked out for the first time from world level.

FAMILY: TILIACEAE Juss.

Corchorus aestuans L.: The chromosome count of the species reveals the presence of diploid cytotype with n = 7 (Fig. 1k) which is in line with the previous reports from India and outside India [32, 37].

Corchorus olitorius L.: The species depicts the presence of n = 7 (Fig. 1l) which is in line with previous reports from India and outside India.

Triumfetta pentandra A. Rich.: Present accession of the species shows n = 8 (Fig. 1m) which corroborates the previous reports from both India and outside India.

Triumfetta rhomboidea Jacq.: Cytological study of the species collected from different regions of Rajasthan shows two cytotypes of n = 8 (Fig. 1n) and n = 16 (Fig. 1o) of which n = 16 is most probably the first tetraploid cytotype from India while n = 8 is already reported from India [21].

Triumfetta rotundifolia Lam.: Present meiotic study of the species depicts the presence of n = 16 (Fig. 1p) which is earlier reported only from India [23].

Meiotic abnormalities

Detailed meiotic course of some species shows abnormal behaviour with the presence of bridges, laggards, interbivalent connections, cytomixis, unoriented bivalents which leads to heterogenous sized pollen grains (Fig. 2a-l). From present study diploid cytotype of *Triumfetta rhomboidae* and *Corchorus aestuans* shows the maximum percentage of cytomixis at different meiotic stages (Table 2).

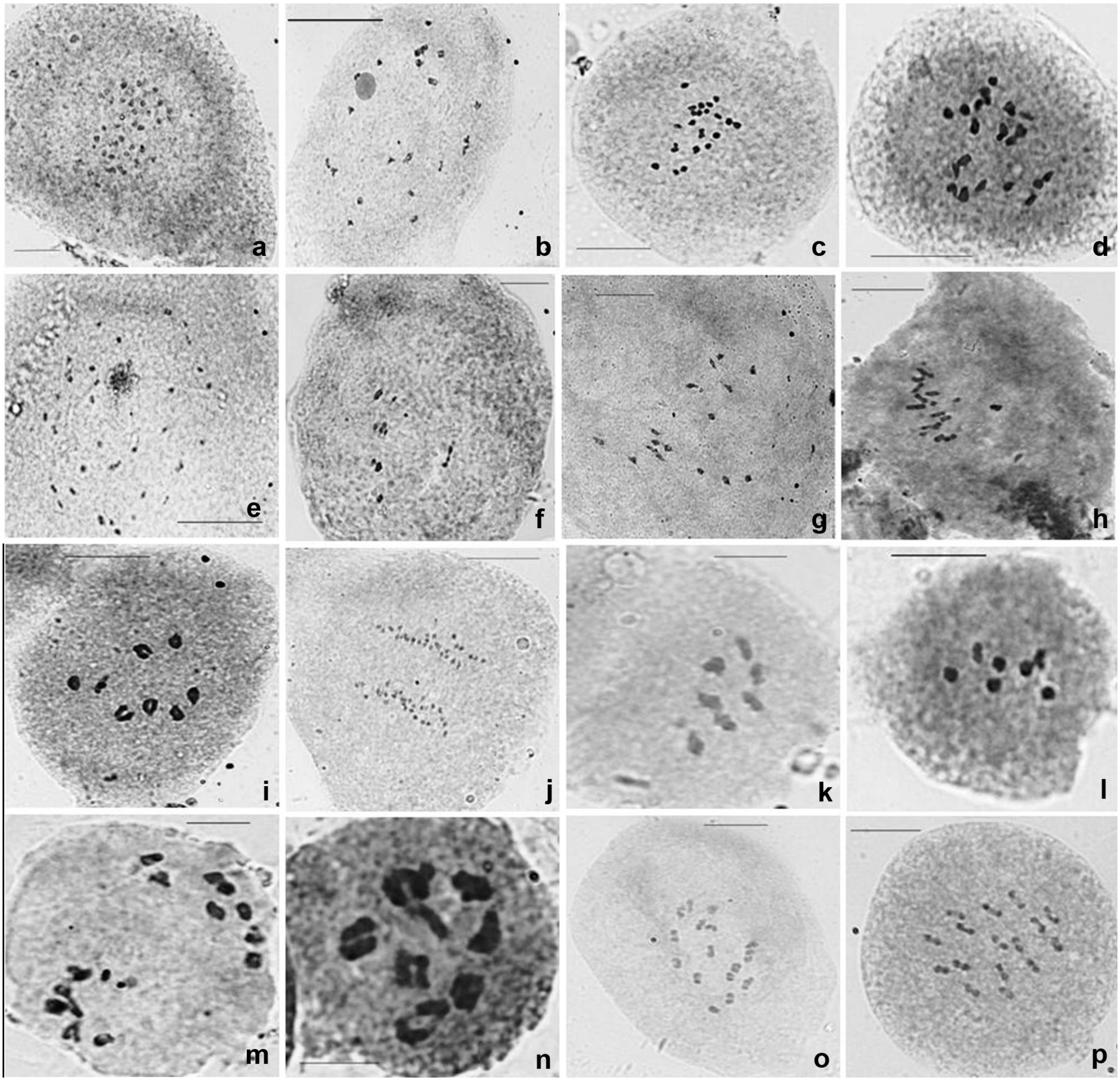

Fig. 1 Dipiction of meiosis: **a** *Abelmoschus moschatus*: PMC at A-I showing 18:18 distribution of chromosomes. **b** *Abutilon pannosum*: PMC at Diakinesis showing 21_{II}. **c** *A. persicum:* PMC at M-I showing 21_{II}. **d** *A. ramosum*: PMC at M-I showing 21_{II}. **e** *Hibiscus lobatus*: PMC at Diakinesis showing 36_{II}. **f** *Sida alba*: PMC at M-I showing 7_{II}. **g** *Sida cordata*: PMC at M-I showing 16_{II}. **h** *Sida ovata*: PMC at M-I showing 14_{II}. **i** *S. rhombifolia*: PMC at M-I showing 8_{II}. **j** *Melhania magnifolia*: PMC at A-I showing 30:30 chromosomal distribution at A-I. **k** *Corchorus aestuans*: PMC at M-I showing 7_{II}. **l** *Corchorus olitorius*: PMC at M-I showing 7_{II}. **m–o** *Triumfetta rhomboidea*: **m** PMC at A-I showing 8:8 distribution of chromosomes. **n** PMC at M-I showing 8_{II}. **o** PMC at M-I showing 16_{II}. **p** *Triumfetta rotundifoila*: PMC at M-I showing 16_{II}

Cytomixis usually lead to the formation of hypo/hyperploid cells which leads to formation of heterogenous sized pollen grains. The present study also depicts the abnormal meiotic behaviour with the presence of laggards (*Abutilon pannosum, Triumfetta rhomboidea*), chromatin bridges (*A. pannosum*) at A-I, early and late disjunction of bivalents (*Abutilon ramosum*), Interbivalent connections (*A. ramosum* and diploid cytotype of *Triumfetta rhomboidea*), unoriented bivalents (*A. ramosum*) (Table 2) and all these abnormalities leads to the abnormal microsporogenesis (Table 3) and results to formation of heterogenous sized pollen grains and decreased pollen fertility.

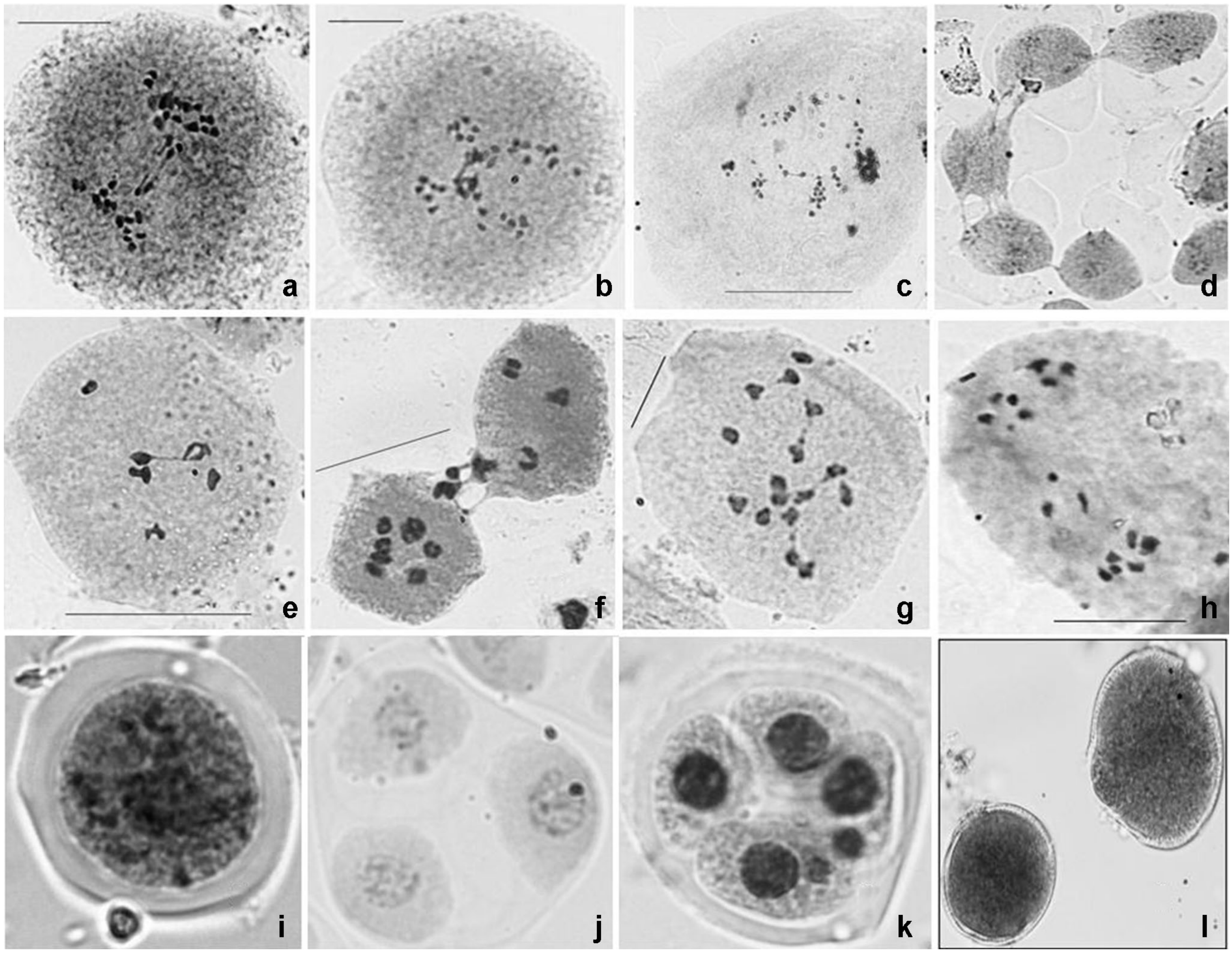

Fig. 2 Dipicting meiotic anomalies and pollen heteromorphism: **a**, **b** *Abutilon pannosum*: PMCs at A-I showing chromatin bridges. **c** *Abutilon ramosum* PMC at M-I showing interbivalent connection. **d–e** *Corchorus aestuans*: **d** PMCs showing chromatin transfer. **e** PMC at M-I showing interbivalent connection. **f–i** *Triumfetta rhomboidea*: **f** PMCs showing cytomixis, **g** PMC showing late bivalent disjunction, **h** PMC at A-I showing chromatin laggards, **i** Monad. **j** Triad. **k** Tetrad with micronuclei. **l** Heterogenous sized pollen grains

Discussion

Chromosome count

FAMILY: MALVACEAE

Abelmoschus: Out of 15 taxonomically known species [34], 8 species/26 cytotypes are cytologically worked out for the genus on worldwide basis. The chromosome number in the genus ranges from 2n = 36, 40, 58, 65, 66, 72, 97, 98, 108, 120, 130 and 132. The genus is dibasic depending on the base numbers x = 18 and 20. From India, all the six taxonomically known species are cytologically worked out.

Abutilon: The genus is comprised of 150 taxonomically known species [30] out of which 83 (55.33 %) species/99 cytotypes are cytologically worked out. It is polybasic in nature depending on the base numbers x = 7, 8 and 9 out of which, x = 7 is the most common one while x = 8, 9 are secondarily evolved base number. In the genus, 39.02 % species shows polyploidy cytotypes with the highest ploidy level is octaploid 2n = 72 depending on the base number x = 8. The most common chromosome number is 2n = 14 found in 31 species while 2n = 32, 56 and 72 are less common. Other known chromosome numbers found in the genus are 2n = 16, 21, 28, 36 and 42. From India, 17 species are taxonomically known out of which 13 are cytologically worked out. The presently worked out three *Abutilon* species shows the hexaploid cytotype without the formation of multivalent. It might be due to allopolyploidy which is already reported in some genus of family Malvaceae as *Gossypium* [36].

Sida: The genus is represented by 150 taxonomically known species world wide, of which 85 species (56 %)/

104 cytotypes are cytologically studied. The chromosome numbers found in the genus are 2n = 12, 14, 16, 18, 20, 22, 28, 32, 34, 42 and 56 of which 2n = 14 is the most common based on x = 7. The genus is polybasic (x = 6, 7, 8 and 9) of which x = 7 is the most common one. From India, all the 8 species are taxonomically known and are cytologically worked out.

Hibiscus: Taxonomically, 250 species of the genus are known for worldwide, of which 129 species (51.6 %)/161 cytotypes are cytologically worked out. The genus is polybasic depending on base numbers x = 7, 8, 9, 11, 12, 15, 17, 19, 20 and 39. The chromosome numbers in the genus shows as a lot of variation ranging from 2n = 20, 22, 24, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 54, 56, 59, 60, 63, 64, 66, 70, 72, 80, 82, 84, 90, 92, 96, 98, 100, 108, 112, 118, 120, 122, 130, 132, 144, 147, 150, 160, 165, 168, 180 and 225.

FAMILY: STERCULIACEAE Vent.

Melhania: Out of 600 taxonomically known species of genus, only 2 species are cytologically worked out with same chromosome number 2n = 60. Depending on the chromosome number of these two species the genus is thought to be monobasic depending on base number x = 10. From India 7 species are taxonomically known of which only 1 species is cytologically worked out.

FAMILY: TILIACEAE

Corchorus: The genus includes 100 taxonomically known species of which 25 species (25 %)/29 cytotypes are cytologically known. From the different chromosome numbers found in the genus (2n = 14, 15, 16, 18, 21, 28 and 36), 2n = 14 is most common followed by 2n = 36 and 28, while all other chromosome number are less common. The genus is polybasic (x = 7, 8 and 9) out of which x = 7 is the primary base number. From India, all the 8 taxonomically known species are cytologically known.

Triumfetta: It is comprises of 159 taxonomically known species out of which 13 species (8.66 %)/22 cytotypes are cytologically studied. The genus is found with dibasic status with x = 8, 10 of which x = 8 is the primary base number. Chromosome number found in different species are 2n = 16, 20, 32, 46, 48, 64 and 84. From India, 8 species are taxonomically studied while 5 species are cytologically studied.

Meiotic abnormalities

Various types of meiotic abnormalities as cytomixis, chromatin stickiness, unoriented bivalents, laggards, bridges have been observed during present study in some species which indicates the existence of intraspecific genetic diversity.

Cytomixis and chromatin stickiness are considered to be the results of genetic factors [13] or environmental factors [25] as well as genetic-environmental interactions [2]. Transfer of chromatin material or chromosomes among the adjacent PMCs occurs through cytomictic channels as well as through cell wall dissolution [14]. Further, the cytomixis leads to the formation of hypo-hyperploid cells [14]. The other meiotic abnormalities observed during present study include chromatin laggards and bridges seen at anaphases and telophases have been occurred due to the abnormal spindle activity. Bivalents that are not lying at the equatorial plate are known as unoriented bivalents. The formation of laggards are attributed to late chiasma terminalisation, late disjunction of chromosomes and stickiness of chromosome ends. The bridge formation is due to the late disjunction [12] followed by terminalisation of chiasmata [26]. Bridge formation may be due to the paracentric inversions. The formation of large sized pollen grains resulting from unreduced 2n pollen grains as has been observed in various angiosperms [5, 20, 31].

The occurrence of sufficient variation of chromosome numbers as well as change of meiotic behavior at intra and interspecific levels of these 15 species calls for further need of the extensive cytological exploration at population basis of members of order Malvales from different phytogeographical areas of India to complete the data bases for Indian Malvales.

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