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Cytomorphological investigations of some species of *Abutilon* Mill. from Punjab

Arneet Gill and Ramneet Kaur*

Department of Botany, Punjabi University, Patiala-147002, Punjab (India)

*Corresponding author: ramneetbawa10@gmail.com

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ABSTRACT

The present studies were conducted to investigate the cytomorphological diversity of the genus *Abutilon* from Punjab. Surveys of the study area revealed presence of four prevalent species. The species were distinct morphologically on the basis of leaf shape, floral diameter, number, shape and colour of mericarps and awn size. The species were identified to be *A. ramosum* (Cav.) Guill. & Perr., *A. theophrasti* Medik., *A. bidentatum* Hochst. ex A. Rich. and *A. indicum* (L.) Sweet. Three species namely *A. theophrasti*, *A. bidentatum* and *A. indicum* showed a gametophytic chromosome count of $n=21$ as determined from male meiotic studies. *A. ramosum* showed the presence of $n=8$ gametophytic chromosome count, which is a first chromosomal number report for the species. The chromosome report of $n=21$ for *A. theophrasti* is a first for India. The course of meiosis in all the species was observed to be normal in majority, however some PMCs showed the presence of abnormalities such as interbivalent connections, laggards, bridge formation, cytotoxicity, vagrants, stickiness and multipolarity etc.

Key Words: *Abutilon*, Morphology, Cytology, Punjab.

INTRODUCTION

Abutilon belongs to family Malvaceae in having hairy body, pentamerous, actinomorphic, complete flower, with monadelphous androecium, syncarpous, multilocular ovary and axile placentation. The genus is however unique due to the absence of epicalyx along with *Sida* and *Herissantia*. *Abutilon* is a large genus with nearly 200 recognised species distributed in tropical and sub-tropical regions of the World (Sivarajan & Pradeep 1996). *Abutilon* species include annuals, perennials, herbs, shrubs or even small trees. India is a recognised biodiversity centre of the world and a total of eighteen *Abutilon* species have been recognised here (Bamber 1916, Sharma & Tiagi 1979, Chowdhery & Wadhwa 1984, Sivarajan & Pradeep 1996, Kumar 2001 and Singh *et al.* 2002).

Abutilon is considered as an important medicinal plant in the folk medicine. It is used for curing leprosy, diabetes, jaundice, piles, ulcers, bronchitis, diarrhoea, inflammation of bladder, fever, gonorrhoea, besides being used for common ailments as cough, cold, etc. (Ahmed *et al.* 1990, Kaushik *et al.* 2009, Khadabadi & Bhajipale 2010, Das *et al.* 2012, Kousalya *et al.* 2014 and Ramar & Ayyadurai 2015). Ten species have been worked out cytologically from India, most of which belong to the Southern peninsula (IPCN 2015). The plant is found growing aplenty in the state of Punjab from where only three species have been worked out cytologically (IPCN 2015). The present study was aimed to identify the species of genus *Abutilon*

found in Punjab and study their morphology and course of male meiosis in details.

MATERIALS AND METHODS

A total of 96 populations of *Abutilon* were collected from different localities of Punjab (Table 1). The plant species were identified from Herbarium of Department of Botany, Punjabi University, Patiala, Punjab.

Morphological study

To identify the species and morphotypes, all collections were evaluated on the basis of morphological characters like habit, leaf shape and size, colour and size of flower, number of stigma, colour, number, shape and size of mericarp and awns.

Cytological study

Cytological investigations were carried out from temporary mounts of young anthers squashed in acetocarmine. Appropriate sized floral buds were fixed in Carnoy's fixative for 24h. Then preserved in 70% alcohol and stored at 4° C for further study. The apparent pollen fertility was estimated through stainability test in glycerol-acetocarmine (1:1) mixture as given by Marks (1954). The photographs were taken from freshly prepared

temporary slides using Nikon-80i Eclipse microscope.

RESULTS AND DISCUSSION

Morphological studies revealed that the species found in present study are perennial shrubs growing wild along the road sides. Morphologically four distinct species were apparent namely, *A. ramosum* (Cav.) Guill. & Perr., *A. theophrasti* Medik., *A. bidentatum* Hochst. ex A. Rich. and *A. indicum* (L.) Sweet.

A. ramosum plants are erect, pubescent with height upto 2m (Fig. 1a). Leaves of the species are simple, alternate, cordate, subtrilobate, 9-nerved with lamina reaching a length of nearly 15cm (Fig. 1b). Plants bear yellow flowers which may be solitary or in racemose panicles. Each flower may be 1.2-1.5cm in diameter (Fig. 1c). Flowers bear six carpels. Fruit is a schizocarp having 6-8 mericarps (Fig. 1d) measuring $0.8 \pm 0.05 \times 0.4 \pm 0.05$ cm in size with nearly 2 mm long awns (Fig. 1e). Fruit shows the presence of stellate hair. Stellate hairs have 5-10 free ends (Fig. 1g). The seeds are heart-shaped, blackish in colour and 3 in each mericarp (Fig. 1f). The observations are in line with the previous studies of Thulin (1999). In previous literature, Parker (1918) and Sivarajan and Pradeep (1996) have reported the presence of 8-10 carpels in *A. ramosum*.

Table 1: List of species of *Abutilon* studied from Punjab.

S. No.	Species	No. of Populations studies	Gametic Chromosome Number (n)	Comments
1.	<i>A. ramosum</i> (Cav.) Guill. & Perr.	3	8	Meiotic course is normal in 1 population and 2 populations showed meiotic abnormalities
2.	<i>A. theophrasti</i> Medik.	24	21	Meiotic course is normal in 23 populations and 1 population showed meiotic abnormalities
3.	<i>A. bidentatum</i> Hochst. ex A. Rich.	19	21	Meiotic course is normal in 16 populations and 3 populations showed meiotic abnormalities
4.	<i>A. indicum</i> (L.) Sweet	50	21	Meiotic course is normal in 32 populations and 18 populations showed meiotic abnormalities

A. theophrasti has simple, erect, pubescent stem and grows upto 2m height (Fig. 1i). Leaves of the plant are alternate, ovate, deeply cordate, long acuminate, 9-nerved at base with lamina upto 15cm in length (Fig. 1j). Flowers are yellow in colour, pentamerous and solitary. Each flower may be 1-1.5cm in diameter and having 12-15 carpels (Fig. 1k). Schizocarps have 12-16 mericarps and turns to black on maturity (Fig. 1l). Mericarps are $0.71 \pm 0.02 \times 0.33 \pm 0.02$ cm in size having 1-2 mm long awn (Fig. 1m). Stellate hairs have 9-11 free

ends (Fig. 1o). The seeds are heart-shaped, blackish to dark brown in colour and 2-3 in each mericarp (Fig. 1n). The observations are in line with the previous records by Spencer and Sankaran (1985).

The plants of *A. bidentatum* are erect, pubescent with upto 2m in height (Fig. 1q). Leaves are alternate, ovate having cordate base, acuminate, pubescent, base 9-nerved with lamina reaching nearly 12cm in length (Fig. 1r). Flowers are pale yellow, pentamerous, 1-1.5cm in diameter and bear 13-16 carpels (Fig. 1s). Schizocarps have 13-18

Table 2: Details of abnormal populations of *Abutilon* collected from different regions of Punjab.

S. No.	Species	Population	Accession Number(PUN)	Abnormalities												
				I	II	III	IV	V	VI	VII	VIII	IX	X	XI		
1.	<i>A. ramosum</i> (Cav.) Guill. & Perr.	Sangrur	59800	+	-	+	+	-	+	-	-	-	-	-	+	
			59801	+	-	-	-	-	-	-	-	-	-	-	-	-
2.	<i>A. theophrasti</i> Medik.	Patiala	59799	+	-	-	+	+	-	-	-	-	+	-	+	
3.	<i>A. bidentatum</i> Hochst. ex A. Rich.	Barnala	59802	-	-	-	+	-	-	-	-	-	-	-	-	
			59804	-	-	-	+	-	+	+	-	-	-	-	-	
		Sangrur	59803	-	+	-	+	-	-	-	-	-	-	-	-	-
4.	<i>A. indicum</i> (L.) Sweet	Patiala	59805	-	-	-	-	+	-	-	-	+	+	-	-	
			59806	+	-	-	-	+	-	-	-	-	-	-	+	
			59810	+	-	-	-	-	-	-	-	-	-	-	-	-
			59811	-	+	-	+	+	-	+	-	-	-	-	-	-
			59812	-	-	-	+	-	-	-	-	-	-	-	-	-
			59813	+	+	-	+	-	-	-	-	-	+	+	+	+
		Barnala	59807	-	-	-	-	-	-	+	-	-	-	-	-	-
			59808	+	+	-	+	-	-	-	-	-	-	-	-	-
			59818	-	-	+	+	-	-	+	-	-	-	-	-	-
		Ludhiana	59814	-	+	-	-	-	-	-	-	-	-	-	-	-
			59820	-	+	-	+	-	-	-	-	-	-	-	-	-
		Mansa	59815	-	-	+	+	-	+	-	-	-	-	-	-	-
		Hoshiarpur	59823	-	-	-	-	-	-	-	-	-	-	+	-	-
		Rupnagar	59819	-	-	-	-	-	+	+	-	-	-	-	-	-

I: Stickiness, **II:** Interbivalent connections, **III:** Secondary associations of chromosomes, **IV:** Vagrant, **V:** Laggards, **VI:** Bridge, **VII:** Cytomixis, **VIII:** Early disjunction, **IX:** Monad, **X:** Dyad, **XI:** Multipolarity, +: Present and -: absent

mericarps that turn light brown on maturity (Fig. 1t). Mericarps are $0.61 \pm 0.07 \times 0.3$ cm in size and having small awn of about 0.6 mm (Fig. 2a). Stellate hairs have 9-11 free ends (Fig. 2c). The seeds are heart-shaped, blackish to dark brown in colour, 2-3 in each mericarp (Fig. 2b). In previous literature, Singh *et al.* (2002) have reported 13-16 mericarps in the schizocarp.

In case of *A. indicum*, the plants are erect and upto 3m in height (Fig. 2e). Leaves are ovate with dentate margin, cordate, 9-nerved with lamina reaching a length of nearly 18cm (Fig. 2f). Flowers are yellow in colour and 2.0-2.5cm in diameter. Flowers bear 15-20 carpels with capitate stigma (Fig. 2g). Schizocarps have 15-20 mericarps (Fig. 2h). Mericarps are $1.4 \pm 0.1 \times 0.8 \pm 0.1$ cm in size with

nearly 1 mm long awns (Fig. 2i). Stellate hairs have 5-6 free ends (Fig. 2k). There are 2-3 seeds in each mericarp. Seeds are heart-shaped and dark brown or black in colour (Fig. 2j). The studies are in line with previous reports by Jain *et al.* (2011) and Gaikwad & Mohan (2011). In previous literature size of leaves was reported upto 12 cm (Jain *et al.* 2011). Sivarajan & Pradeep (1996) and Sahoo *et al.* (2011) have reported the presence of 17-20 and 15-22 mericarps in the schizocarp respectively.

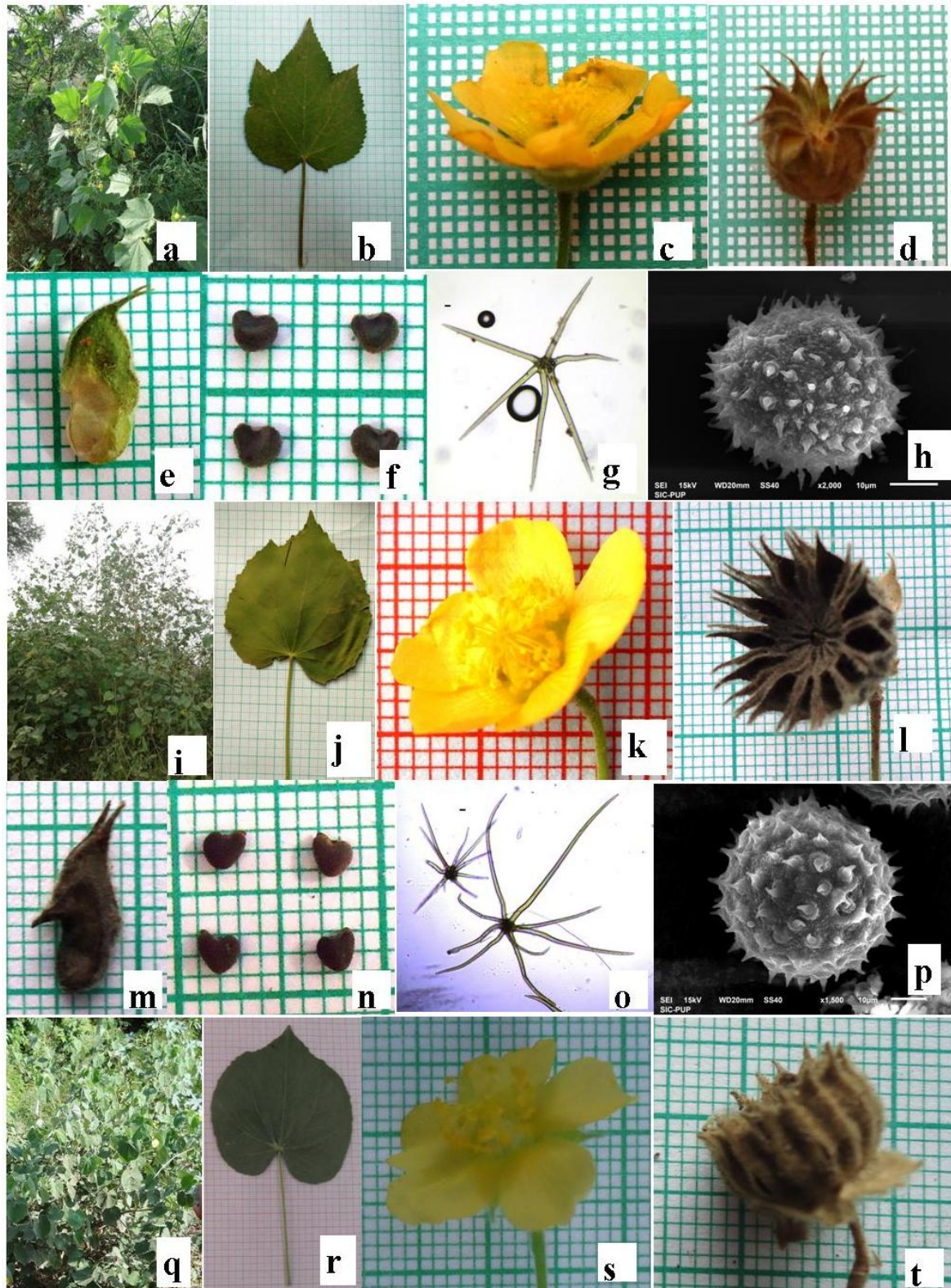


Fig. 1: a) Field photograph of *A. ramosum* b) leaf c) flower d) Fruit (Schizocarp) e) Mericarp f) Seeds g) Stellate hair h) Pollen grain i) Field photograph of *A. theophrasti* j) leaf k) flower l) Fruit (Schizocarp) m) Mericarp n) Seeds o) Stellate hair p) Pollen grain q) Field photograph of *A. bidentatum* r) leaf s) flower t) Fruit (Schizocarp)

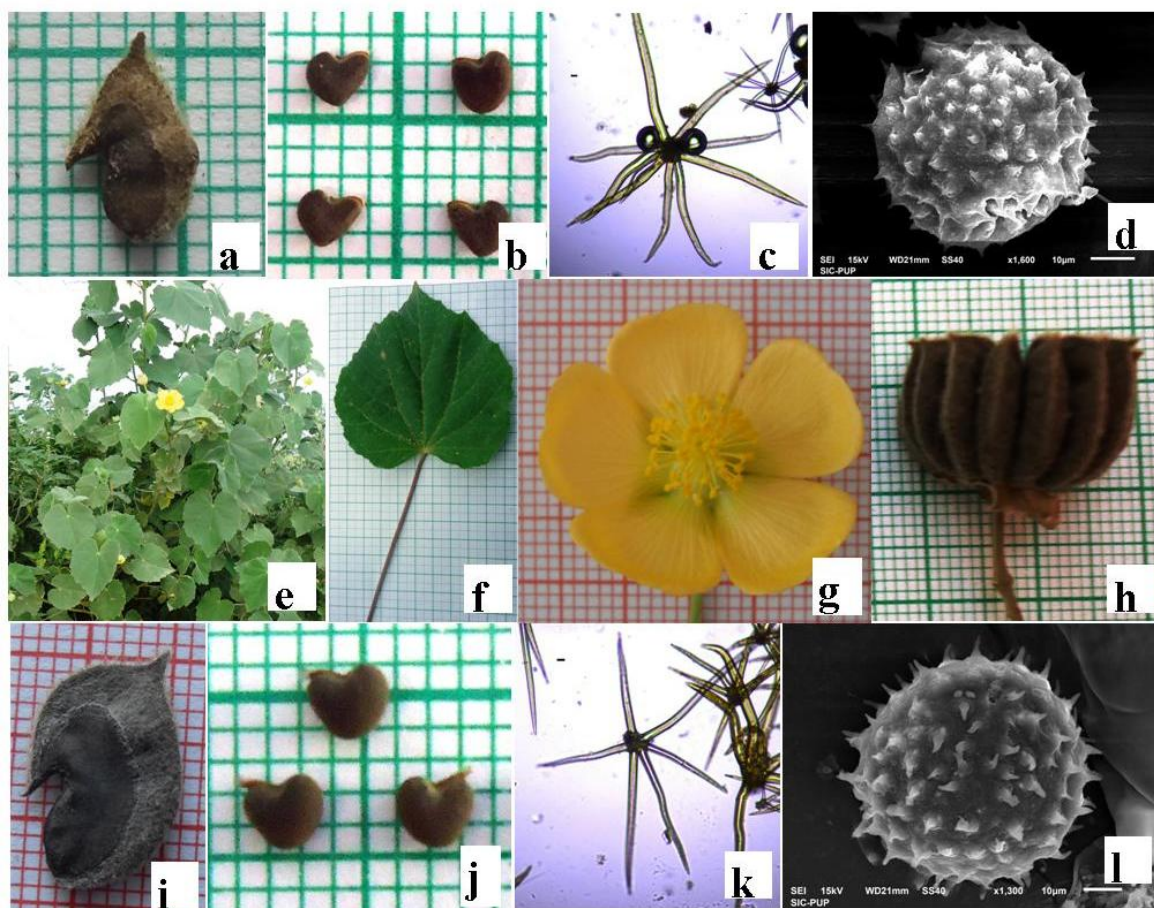


Fig. 2: a) Mericarp b) Seeds c) Stellate hair d) Pollen grain e) Field photograph of *A. indicum* f) leaf g) flower h) Fruit (Schizocarp) i) Mericarp j) Seeds k) Stellate hair l) Pollen grain

Previously, *A. grandifolium* (Willd.) Sweet has been reported to be prevalent in the study area (Sharma & Bir 1978), however the authors were presently unable to spot the species from the reported locations. This may be an indication of loss of biodiversity in the state of Punjab.

Cytological studies

All populations of *A. theophrasti*, *A. bidentatum* and *A. indicum* revealed the haploid chromosome number of $n=21$. All the three populations of *A. ramosum* collected from Lehragaga (Sangrur) showed the presence of $n=8$ (Fig. 3a, 3b, 3c) as haploid chromosome number (Table 1). The present count of $n=8$ is new report for *A. ramosum*. *A. theophrasti* with $n=21$ (Fig. 3j, 3k) was reported for the first time from India and present count was in line with the previous records from outside India (Skovsted 1935, 1941; Ford 1938; Podlech & Dieterle 1969; Uhríkova & Majovsky 1980; Markova 1982; Rudyka 1986; Markova & Goranova 1993 and Shatokhina 2006). *A. bidentatum* with gametic chromosome count of $n=21$ (Fig. 4f, 4g) was reported previously from the same study area by Bir & Sidhu (1978, 1979, 1980) and Sidhu (1979). Gametic chromosome number of

A. indicum ($n=21$) (Fig. 4n, 4o) was in line with the previous records by Bir & Sidhu (1980), Krishnappa & Munirajappa (1983), Carr (1985), Husain *et al.* (1988), Munirajappa & Krishnappa (1993), Cheng & Tsai (1999).

Meiotic abnormalities like interbivalent connections (Fig. 5a), laggards (Fig. 5e), secondary association of chromosomes (Fig. 5b), bridge formation (Fig. 5f), cytomixis (Fig. 5g), vagrants (Fig. 5c), stickiness (Fig. 5d), multipolarity (Fig. 5h), monad (Fig. 5i) and dyad formation (Fig. 5j) were observed in some of the populations of *A. indicum* from Barnala, Patiala, Ludhiana, Mansa, Rupnagar and Hoshiarpur districts. The populations of *A. bidentatum* from Sangrur and Barnala showed interbivalent connections (Fig. 4h), laggards (Fig. 4j), bridge formation (Fig. 4k), vagrants (Fig. 4i), monad and dyad formation (Fig. 4l). One population of *A. theophrasti* from Patiala also showed meiotic abnormalities like secondary association of chromosomes (Fig. 3l), laggards (Fig. 3n), vagrants (Fig. 3m), monad (Fig. 4a), monad with one micronuclei (Fig. 4b) and polyad formation (Fig. 4c, 4d). Aberrations in meiotic course were also recorded in *A. ramosum*, which include secondary association of chromosomes (Fig. 3e), bridge formation (Fig. 3g), vagrants (Fig.

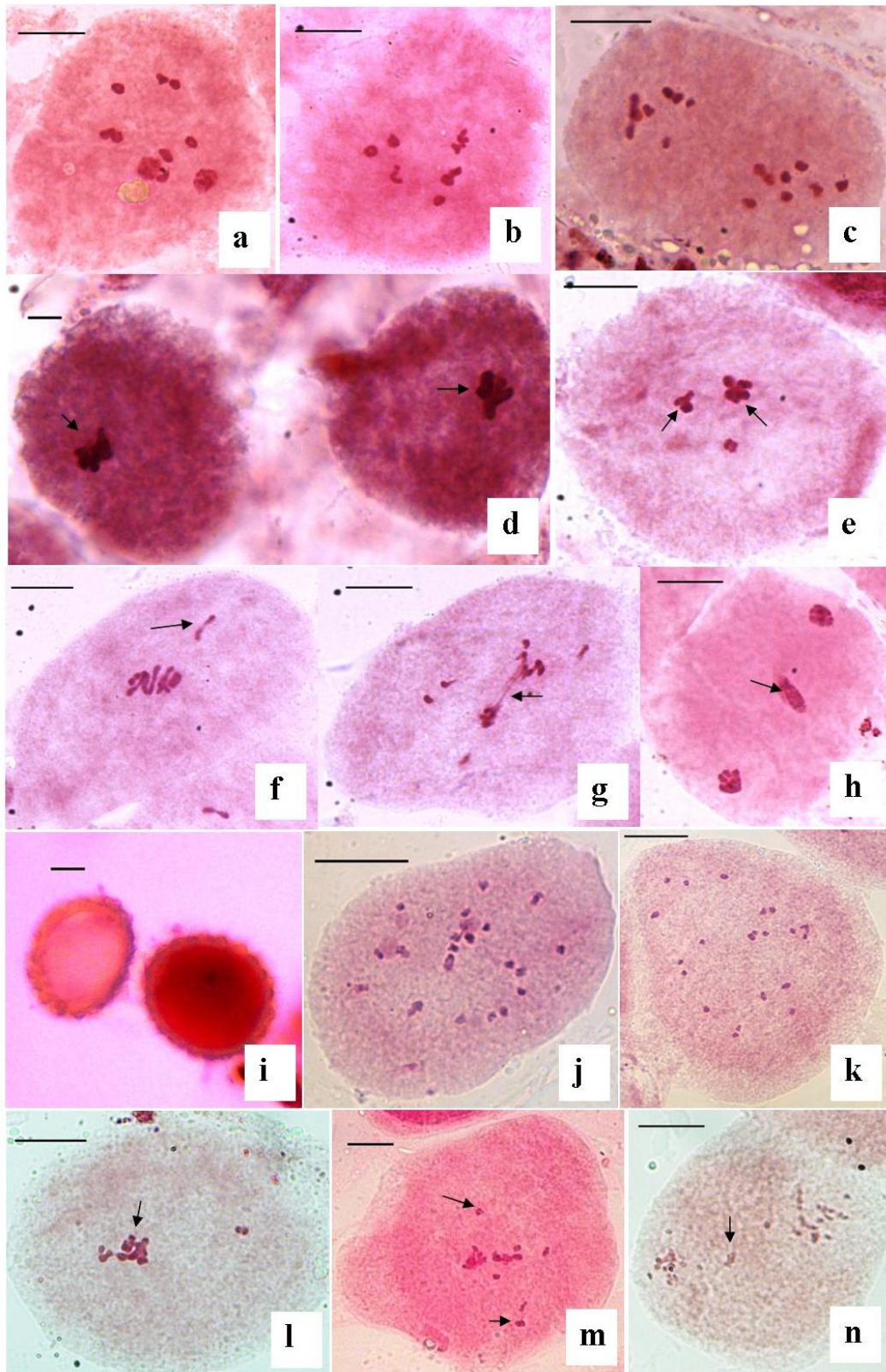


Fig. 3: a) PMC showing 8 II at Diakinesis b) PMC showing 8 II at M-I c) PMC showing 8:8 distribution of chromosomes at A-I d) PMC's showing stickiness e) PMC showing secondary associations of chromosomes f) PMC showing vagrant g) PMC showing bridge formation h) PMC showing multipolarity at T-I i) Fertile and sterile pollen grain j) PMC showing 21 II at Diakinesis k) PMC showing 21 II at M-I l) PMC showing secondary associations of chromosomes m) PMC showing early disjunction n) PMC showing laggards. (Bar = 10 μ m)

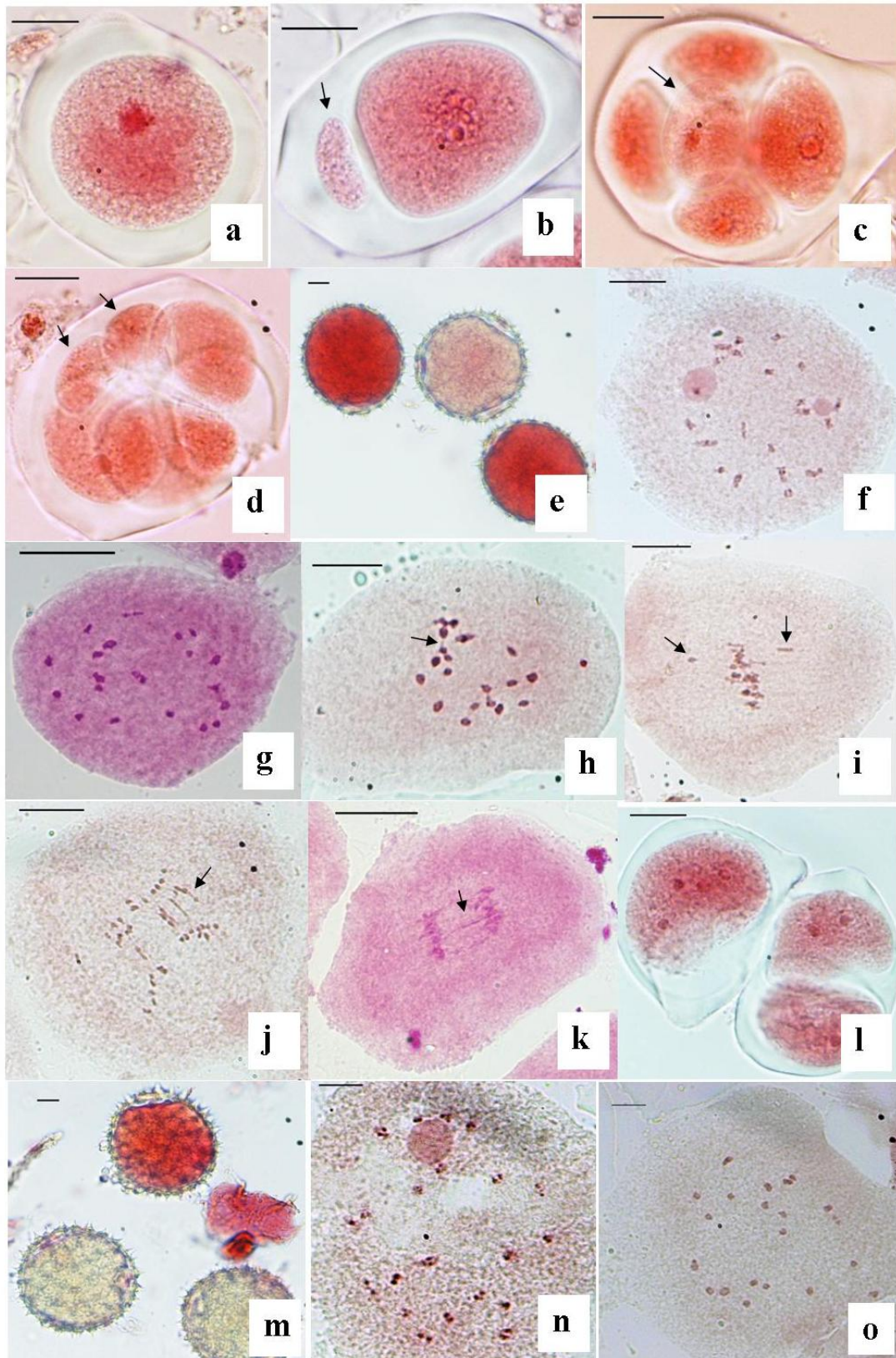


Fig. 4: a) Monad b) Monad with one micronuclei c) Polyad d) Polyad e) Fertile and sterile pollen grain f) PMC showing 21 II at Diakinesis g) PMC showing 21 II at M-I h) PMC showing interbivalent connections i) PMC showing early disjunction j) PMC showing laggards k) PMC showing bridge formation l) Monad and Dyad m) Heterogeneous sized fertile and sterile pollen grain n) PMC showing 21 II at Diakinesis o) PMC showing 21 II at M-I. (Bar = 10 μ m)

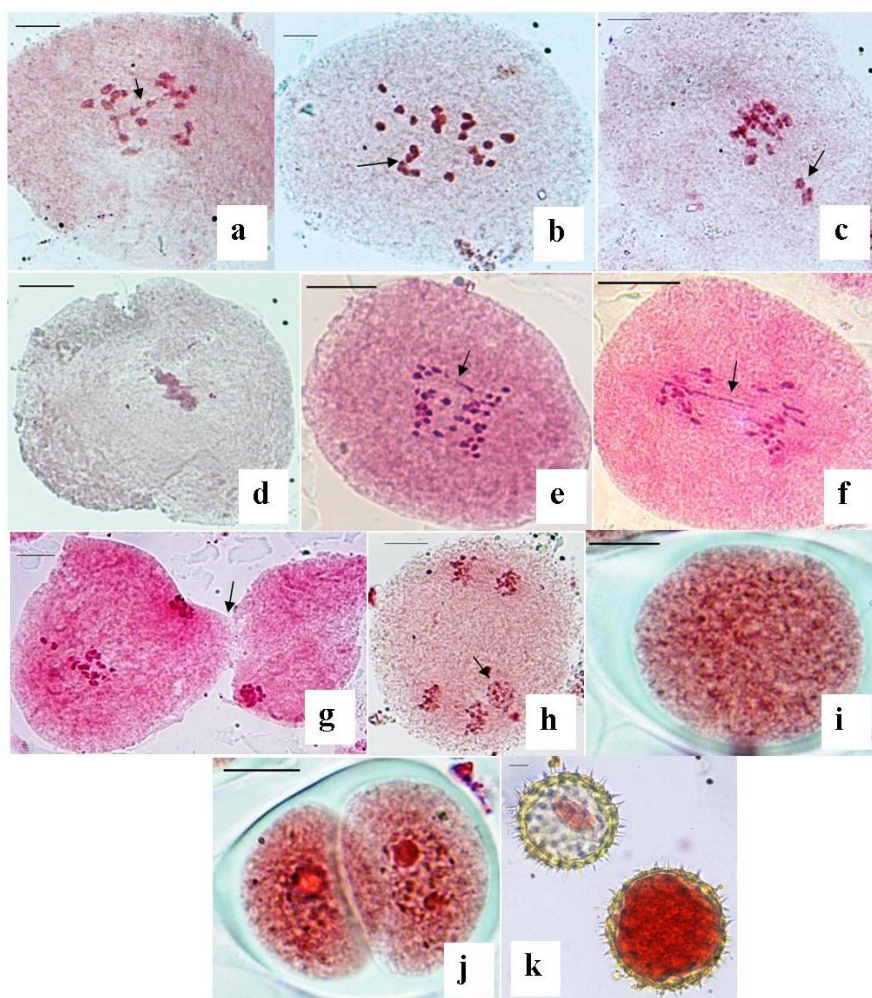


Fig. 5: a) PMC showing interbivalent connections b) PMC showing secondary associations of chromosomes c) PMC showing vagrant d) PMC showing stickiness e) PMC showing laggards f) PMC showing bridge formation g) PMC showing cytomixis h) PMC showing multipolarity at T-II i) Monad j) Dyad k) Heterogeneous sized fertile and sterile pollen grain. (Bar = 10 μ m)

3f), stickiness (Fig. 3d) and multipolarity (Fig. 3h) (Table 2). Meiotic abnormalities may be responsible for pollen sterility and heterogenous pollen size in the species (Fig. 3i, 4e, 4m, 5k).

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