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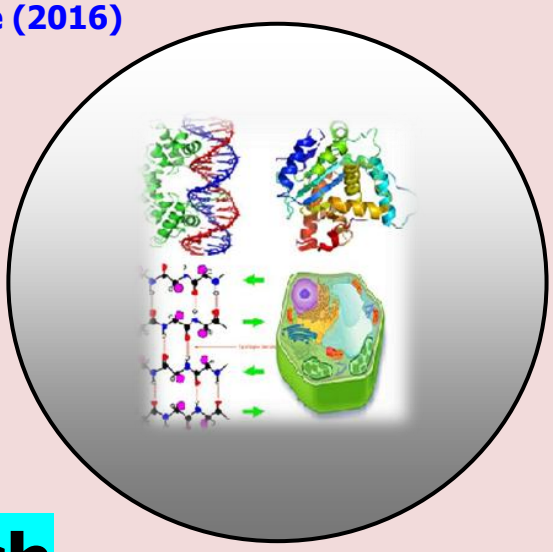

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Evaluation of Cytological Stability in two Medicinally Important Herbs of Caryophyllales from Thar Desert, Rajasthan

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ABSTRACT

The present work deals with evaluation of cytological stability in Gisekia pharnaceoides Linn. and Corbichonia decumbens (Forssk.) Exell, belongs to order Caryophyllales. Both the plants were placed earlier in family Molluginaceae but their present status is changed as Gisekia is now placed in family Gisekiaceae while Corbichonia in Lophiocarpaceae of Caryophyllales, their placement always remained disputed. Both the plants are medicinally important as Gisekia is used to cure swelling, some female disorders, is also used as aperient, purgative and as an anthelmintic. Corbichonia is used to cure kidney stone and also used as a tonic in gonorrhoea, as an antioxidant, anti-inflammatory and antiulcer agent. These plants have been least studied cytologically. The reports shows that Gisekia posses n=9 as base no. Spontaneous polyploidy is common in this plant as it reveals 2n=36, 72 and 108 chromosomes. Our findings confirms 2n=4x=36 in Gisekia, while in Corbichonia it is 2n=18. Karyotype analysis confirms their symmetrical nature supporting stability and least variations.

Key words- Karyotype, Cytological stability, Medicinal plants, Chromosomes, leggards and Centromeric Index.

INTRODUCTION

Chromosomal features are helpful in elucidating phylogenetic affinities and evolutionary development as they are indicators of appropriate classification of several plants (Jones, 1978). The result of chromosomal studies may also be useful in plant taxonomy and phylogenetic analysis (Sudarshana *et al.,* 2015). Knowledge of chromosome structure has played crucial role in the improvement of medicinally important plant species and has far reaching implications (Samaddar *et al.,* 2012). According to Gill and Singhal (1998) chromosomal surveys involving the determination of chromosome numbers and meiotic behaviour are of immense importance in understanding the cytogenetic constitution of species, relationships among taxa and to provide a base for future improvement programmes. Chromosome studies are valuable determinants in studying evolution (Gupta *et al.,* 2014).The data on chromosome number and karyological analysis are prerequisites to overall understanding of a genome and its genetic amelioration through several approaches of crop breeding (Behera *et al.,* 2010). Chromosomes are the carriers of genetic information provided an impetus for their studies since the establishment of the chromosomal theory of inheritance in the second decade of nineteenth century (Badr and Gasim, 1992). *Gisekia pharnaceoides* is an important medicinal herb of Rajasthan. These are

annual or perennial herbs or subshrubs, having simple leaves, mostly terminal inflorescences, small flowers etc. The base number of x=9 has been reported for Molluginaceae. Nakai (1942) was the first to create the monotypic family Gisekiaceae. Seed characters support the independence of *Gisekia* (Gisekiaceae) in a family of its own (Hassan *et al.,* 2005). It is a common creeping and well branched annual herb of medicinal values found in sand dunes, is used for treating various ailments (Arora and Saini, 2016, 2017). Several interesting features i.e. presence of rosette crystals, secondary growth, lateral root, large vessels, perivascular fibers support weed like growth (Arora and Saini, 2017). *Corbichonia decumbens* (Lophiocarpaceae) is a prostrate, glabrous, succulent and annual herb found almost throughout the India. This family is comprised of about six species, distributed in africa, mainly in the southwest, and southwestern Asia (Endress *et al.,* 1993). This plant is used to cure kidney stone problems and gonorrhoea (Uma *et al.,* 2013). It is also used as an antioxidant, anti-inflammatory, antiulcer, antimicrobial, and antinociception (Arora and Saini, 2017). The aim of the present study was to evaluate the detailed mitotic and meiotic behaviour of these plant using 3 accessions of each. The chromosomes number earlier reported in root tip mitosis was also confirmed.

MATERIAL AND METHODS

Germplasm of *Gisekia* was collected from Mandor, Mathania and Ossian (Jodhpur) while germplasm of *Corbichonia* was collected from Beriganga, Machia safari and Bheem-bhadak of Jodhpur (Rajasthan). Seed viability was tested by Triphenyl tetrazolium chloride (TTC) viability test. Seed germination was done in petri-dishes containing moist Whatmann No.1 filter paper. Seeds were treated with 15ppm of GA (Gibberellic acid) for 24 hours. Untreated (controlled) seeds were not responding towards germination at room temperature. Treated seed were incubated at room temperature for 48 hours in dark for germination. Young and healthy root tips (0.5 to 1.5 cm) were excised between 7.30 - 8.30 am, thoroughly washed in water and fixed in FAA (formaldehyde -acetic acid -ethanol). For mitotic studies meristematic tips (0.1/0.2mm) were excised and squash were made using acetocarmine. They were observed and analyzed in Olympus BX-60 microscope fitted with 10x, 20x, 40x and 100x objectives. For meiotic studies, young flower buds of appropriate sizes were collected from the field from healthy plants growing under natural conditions then fixed in a freshly prepared fixative. After fixation the buds were transferred to 70% ethanol and stored in refrigerator for future use. Meiotic squashes were prepared by using the young and developing anthers in a drop of 1% acetocarmine.
Microphotographs for both mitotic and meiotic studies were taken using Dewinter Digi 1400 camera. For karyotype analysis individual chromosomes were cut from well spread metaphasic complements, arranged in descending order of their length from left to right and grouped to form homologous pairs on the basis of gross morphology and centromeric position. Centromeric Index (F %) was calculated to observe the stability in germplasm of both the plants.

RESULTS AND DISCUSSION

***Gisekia pharnaceoides* Linn**

Only few reports are available on chromosomal studies in these plants. Within Molluginaceae both in *Mollugo* and *Gisekia* a basic chromosome number of n=9 has been reported (Sharma and Ghosh, 1968). *Gisekia* is occasionally eaten as a vegetable in Somalia, Kenya and Tanzania. In India it is treated as a weed and used as an emergency food in some parts. It reveals many medicinal properties, i.e. in East Africa the whole plant is eaten as a general strength restorative, e.g. after miscarriage. In India, it is used as a taenicide.
For Karyology, seeds of the diploid plant were collected and germinated on moist filter paper lined petri dish at approximately 34°C. Freshly grown root tips (0.5-1cm) were cut off between 8 to 9 am to obtain mitotic metaphases and to determine karyotype characteristics, i.e. chromosome number, chromosome length and total length of all chromosomes. Firstly these root tips were placed in colchicine solution (0.1%) for 3 hours. The fixed tips were then washed thoroughly in distilled water and meristems were hydrolysed in 1M hydrogen chloride (HCl) for about 1-2 min at room temperature. 1 to 2 mm length from the tips were excised and placed on clear glass slides with acetocarmine as a stain. Squashes were made for cytological studies. The scattered cells showing chromosomal complements and cell division were photographed using (Olympus BX-60) phase contrast fluorescent microscope fitted with digital camera. Metaphases were screened using Photoshop 7.0 software to prepare the karyotype. The method for karyotypic analysis followed Li and Chen (1985) and the karyotype classification was based on Stebbins (1971). Both the numbers and the character of chromosome in mitosis are the most persuasive checking standards for identifying ploidy level.

Table 1. C.I. values in various accession of *Gisekia pharnaceoides* Linn.

JMT		Centromeric index (F %)	JOS		Centromeric index (F %)	JMAU		Centromeric index (F %)
Length of p arm	Length of q arm	Nature of constriction	Length of p arm	Length of q arm	Nature of constriction	Length of p arm	Length of q arm	Nature of constriction
0.3	0.3	50 (M)	0.3	0.4	42.85(NM)	0.3	0.4	42.85(NM)
0.2	0.4	33.33 (NSM)	0.2	0.4	33.33 (NSM)	0.35	0.35	50 (M)
0.25	0.35	41.66 (NM)	0.25	0.35	41.66 (NM)	0.2	0.4	33.33 (NSM)
0.2	0.4	33.33 (NSM)	0.25	0.35	41.66 (NM)	0.2	0.5	28.57 (NSM)
0.2	0.4	33.33 (NSM)	0.25	0.35	41.66 (NM)	0.2	0.5	28.57 (NSM)
0.15	0.35	30 (NSM)	0.2	0.4	33.33 (NSM)	0.2	0.4	33.33 (NSM)
0.25	0.25	50 (M)	0.2	0.4	33.33 (NSM)	0.2	0.4	33.33 (NSM)
0.15	0.35	30 (NSM)	0.3	0.3	50 (M)	0.3	0.3	50 (M)
0.2	0.3	40 (NM)	0.2	0.4	33.33 (NSM)	0.3	0.3	50 (M)
0.2	0.3	40 (NM)	0.2	0.4	33.33 (NSM)	0.25	0.35	41.66 (NM)
0.25	0.25	50 (M)	0.2	0.4	33.33 (NSM)	0.2	0.4	33.33 (NSM)
0.15	0.25	37.5 (NSM)	0.25	0.25	50(M)	0.3	0.3	50 (M)
0.15	0.25	37.5 (NSM)	0.2	0.3	40 (NM)	0.2	0.3	40 (NM)
0.15	0.25	37.5 (NSM)	0.2	0.3	40 (NM)	0.2	0.3	40 (NM)
0.1	0.3	25 (SM)	0.2	0.3	40 (NM)	0.2	0.3	40 (NM)
0.1	0.3	25 (SM)	0.2	0.3	40 (NM)	0.2	0.3	40 (NM)
0.2	0.2	50 (M)	0.2	0.3	40 (NM)	0.2	0.3	40 (NM)
0.2	0.2	50 (M)	0.1	0.4	20 (NSM)	0.2	0.3	40 (NM)
0.2	0.2	50 (M)	0.2	0.3	40 (NM)	0.15	0.35	30 (NSM)
0.15	0.15	50 (M)	0.2	0.3	40 (NM)	0.2	0.3	40 (NM)
0.15	0.15	50 (M)	0.2	0.3	40 (NM)	0.25	0.25	50 (M)
0.1	0.2	33.33(NSM)	0.1	0.3	25 (SM)	0.2	0.3	40 (NM)
0.1	0.2	33.33(NSM)	0.2	0.2	50 (M)	0.25	0.25	50 (M)
0.1	0.2	33.33(NSM)	0.2	0.2	50 (M)	0.1	0.4	20 (NSM)
0.1	0.2	33.33(NSM)	0.2	0.2	50 (M)	0.2	0.2	50 (M)
0.1	0.2	33.33(NSM)	0.2	0.2	50 (M)	0.2	0.3	40 (NM)
0.15	0.15	50 (M)	0.2	0.2	50 (M)	0.1	0.3	25 (SM)
0.1	0.2	33.33(NSM)	0.15	0.25	37.5(NSM)	0.2	0.2	50 (M)
0.15	0.15	50 (M)	0.15	0.25	37.5(NSM)	0.1	0.3	25 (SM)
0.1	0.2	33.33(NSM)	0.1	0.3	25 (SM)	0.2	0.2	50 (M)
0.15	0.15	50 (M)	0.2	0.2	50 (M)	0.2	0.2	50 (M)
0.1	0.2	33.33(NSM)	0.15	0.25	37.5(NSM)	0.1	0.3	25 (SM)
0.1	0.2	33.33(NSM)	0.15	0.15	50 (M)	0.1	0.3	25 (SM)
0.1	0.2	33.33(NSM)	0.1	0.2	33.33(NSM)	0.1	0.3	25 (SM)
0.15	0.15	50 (M)	0.1	0.2	33.33(NSM)	0.1	0.1	50 (M)
0.1	0.1	50 (M)	0.1	0.2	33.33(NSM)	0.1	0.1	50 (M)

Key: C.I. (Centromeric Index), NSM (Nearly Sub-Median), M (Metacentric), SM (Sub-Metacentric), NM (Nearly Median)

The centromeric index was calculated for all accessions and the chromosomes were classified according to the centromeric position as, metacentric-M (F% between 50 and 37.5), submetacentric-SM (F% between 37.5 and 25) according to Levan *et al*., (1964). The criterion proposed by Stebbins (1971) and Zarco (1986) was used for the karyotypic symmetry. It was experienced that the high level of chromosomal condensation and similarities in chromosomal sizes hindered the identification of morphologic traits. Visualization of satellite in one pair of chromosome in one accessions of *Gisekia pharnaceoides* was however could be possible.

Temporary squash preparations of young healthy root tip cells show 2n=4x=36 chromosome in *Gisekia*, that is in accordance with earlier findings, it reveals x=9. Most of the chromosomes show metacentric and submetacentric nature of their primary constriction, revealing stability. Length of short arm varies between 0.1-0.3mm, while the length of long arm varies between 0.1-0.5mm. Deviation from symmetry to asymmetry was small that is revealed by total length of chromosomes. Polyploidy most probably is facilitating habitat adjustments rather than genome arrangements. Very small rearrangements do not cause any overall evolutionary make over. The karyotypic homozygosity is directly linked with symmetric nature. The tetraploid cytotypes exhibited normal meiotic behaviour characterized by normal chromosome pairing and regular segregation of chromosomes. The 4x plant grows vigorously and possessed profuse branching and leaves. The photomicrographs show normal chromosomal segregation and distribution. Studies on behaviour of male meiosis were comparatively difficult as the anthers were of much reduced size. No cell plate formation was observed during the separation of daughter cells. Formation of constriction from lateral sides of cell is clearly visible during early telophase, that ultimately forms two daughter cells with well defined wall (Fig.1.-10, 11). Karyotypes for each accession was prepared and formula of each was made using data analysed i.e. $2n=36=L_2^{M}+L_3^{SM}+M_7^{M}+M_7^{SM}+S_7^{M}+S_{10}^{SM}$, $L_5^{M}+L_6^{SM}+M_{15}^{M}+M_6^{SM}+S_1^{M}+S_3^{SM}$, $L_6^{M}+L_6^{SM}+M_{15}^{M}+M_7^{SM}+S_2^{M}$ (Table-1).

Table 2. C.I. values in various accession of *Corbichonia decumbens* (Forssk.) Exell

JBG		Centromeric index (F %)	JBB		Centromeric index (F %)	JMBP		Centromeric index (F %)
Length of p arm	Length of q arm	Nature of constriction	Length of p arm	Length of q arm	Nature of constriction	Length of p arm	Length of q arm	Nature of constriction
0.2	0.4	33.33 (NSM)	0.2	0.4	33.33 (NSM)	0.2	0.4	33.33 (NSM)
0.3	0.3	50 (M)	0.3	0.3	50 (M)	0.3	0.3	50 (M)
0.2	0.4	33.33 (NSM)	0.3	0.3	50 (M)	0.2	0.4	33.33 (NSM)
0.3	0.3	50 (M)	0.2	0.4	33.33 (NSM)	0.3	0.3	50 (M)
0.2	0.3	40 (NM)	0.2	0.3	40 (NM)	0.2	0.3	40 (NM)
0.2	0.3	40 (NM)	0.2	0.3	40 (NM)	0.2	0.4	33.33 (NSM)
0.2	0.3	40 (NM)	0.2	0.3	40 (NM)	0.2	0.3	40 (NM)
0.1	0.4	20 (NSM)	0.2	0.3	40 (NM)	0.2	0.3	40 (NM)
0.2	0.3	40 (NM)	0.2	0.2	50 (M)	0.2	0.2	50 (M)
0.2	0.3	40 (NM)	0.2	0.3	40 (NM)	0.25	0.25	50 (M)
0.2	0.2	50 (M)	0.2	0.2	50 (M)	0.2	0.2	50 (M)
0.2	0.2	50 (M)	0.2	0.2	50 (M)	0.1	0.3	25 (SM)
0.2	0.2	50 (M)	0.2	0.2	50 (M)	0.2	0.2	50 (M)
0.2	0.2	50 (M)	0.1	0.3	25 (SM)	0.2	0.2	50 (M)
0.1	0.3	25 (SM)	0.2	0.2	50 (M)	0.2	0.2	50 (M)
0.2	0.2	50 (M)	0.2	0.2	50 (M)	0.1	0.3	25 (SM)
0.15	0.15	50 (M)	0.15	0.15	50 (M)	0.15	0.15	50 (M)
0.1	0.1	50 (M)	0.15	0.15	50 (M)	0.15	0.15	50 (M)

Key: C.I. (Centromeric Index), NSM (Nearly Sub-Median), M (Metacentric), SM (Sub-Metacentric), NM (Nearly Median)

For meiotic study, young flower buds were selected to squash anthers. Crossing over between bivalents (Diakinesis) in prophase-I (Fig.1.-13) was clearly observed. Metaphase-I cells exhibited stickiness and clumping of chromosomes. Laggards at Anaphase-I were able to migrate to the poles. Anaphase-II showed normal behaviour of segregation. Ring formation was observed in late anaphase-2 (Fig.1.-22). Telophase-1and Telophase-2 was found to be normal in most of the pollen mother cell.

***Corbichonia decumbens* (Forssk.) Exell**

This plant shows similar morphogenetic features as those were observed in *Gisekia.* Anthers are comparatively bigger in size, as were meiocytes. The base no. is 9 and 2n=18 was observed, that is in confirmation with very few earlier reports.

As far as karyology is concerned it is the first report on this genus. Length of short arm varies from 0.1-0.3mm while length of long arm varies from 0.1-0.4mm. Most of the chromosomes are of meta and sub-metacentric nature, again showing symmetric behaviour. Clumping is very common in prophase. Anaphasic spindles are short, the most probable reason may be clumping and stickiness of chromosomes that hinders segregation pattern. Karyotype for each accession was prepared to make formula i.e. 2n=18= $L_2^{M}+L_2^{SM}+M_{10}^{M}+M_2^{SM}+S_2^{M}$, $L_2^{M}+L_2^{SM}+M_{11}^{M}+M_1^{SM}+S_2^{M}$, $L_2^{M}+L_3^{SM}+M_9^{M}+M_2^{SM}+S_2^{M}$ (Table-2). All the 3 accessions had symmetric Karyotype that consisted of metacentric and submetacentric chromosomes. Diplotene chromosomes were clearly visible, they were more condensed. Stickiness was observed in almost all dividing stages. Meiosis was completely of normal behaviour, but minor abnormality was observed at anaphase II, showing few leggards. Spindle mechanism might be disturbed here due to stickiness of chromosomes.

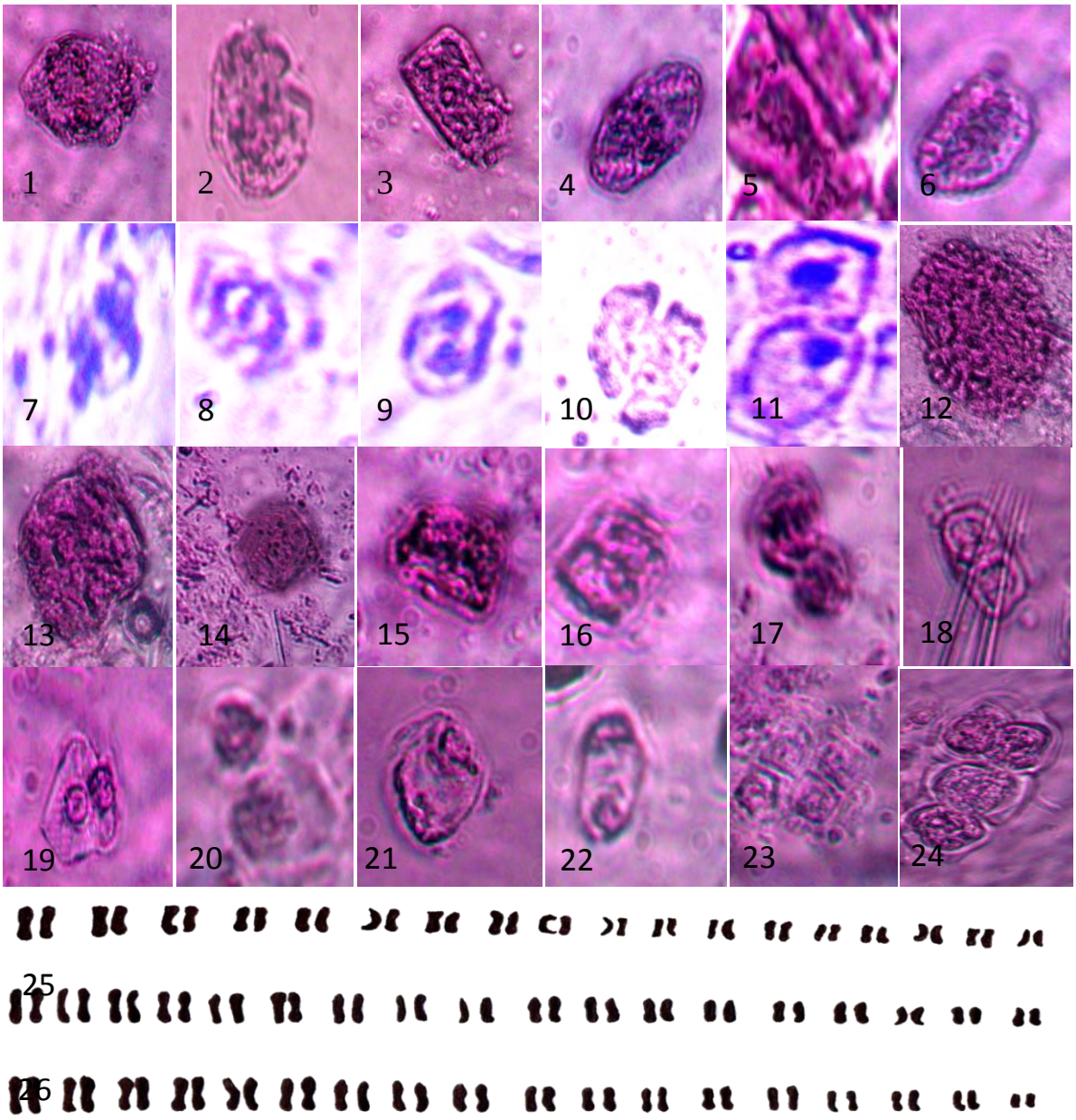

Figure 1. Mitotic behaviour in *Gisekia Pharnaceoides* Linn. Prophase (1-3), Metaphase (4-6), Anaphase (7-8), Telophase (9-11). Meiotic behavior in *Gisekia Pharnaceoides* Linn. Prophase-I (12-14), Metaphase-I (15-16), Anaphase-I (17), Telophase-I (18-20), Anaphase-II (21-22), Telophase-2 (23-24), Karyotypes of various accession of *Gisekia pharnaceoides* Linn. (25-27).

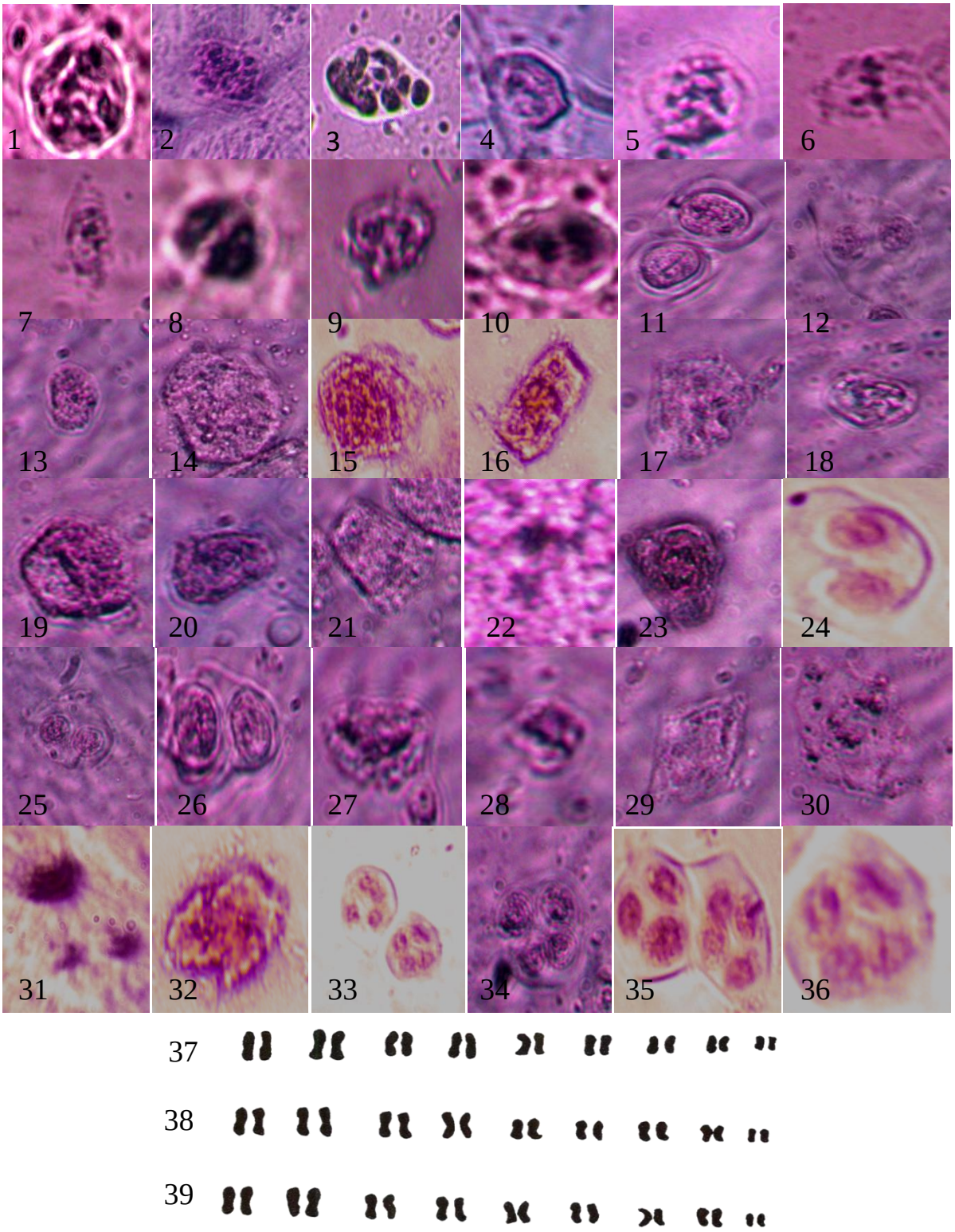

Figure 2. Mitotic behavior in *Corbichonia decumbens* (Forssk.) Exell Prophase (1-3), Metaphase (4-7), Anaphase (8-10), Telophase (11-12). Meiotic behaviour in *Corbichonia decumbens* (Forssk.) Exell; Prophase-I (13-18), Metaphase-I (19-20), Anaphase-I (21-23), Telophase-I (24-26), Metaphase-II (27-28), Anaphase-II (29-32), Telophase-II (33-36), Karyotypes of various accession of *Corbichonia decumbens* (Forssk.) Exell (37-39).

CONCLUSIONS

Gisekia is a spontaneous tetraploid with little advancement towards asymmetry. Biological evolution may raise level of ploidy further, it is supported by weed nature of the plant. This phenomenon showed that a plant with higher ploidy level may be advanced than those with lower ploidy levels. From this study we can conclude that the tetra-ploid genome got rearranged. Detection of low variations between the karyotype of the representatives may help in the identification of the accessions in the germplasm bank. Anyhow, presence of a satellite, ring formation, stickiness, leggards, disturbed spindle mechanism etc. are some of the features that may attract attention for further research on advance level. In overall the behaviour of chromosomes was normal to produce progeny. Similarities in karyotypes may be attributed to genetic stability. Colchicine treatment had not affected the degree of cytological stability.

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