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Cytological Studies of Irregular Meiotic Behavior on Some Medicinal Plants: in Al-Anbar, Iraq.

Naji Swadi Nasser*
Department of Biology, Women Education College, Al-Anbar University, Al-Anbar, Iraq.

Abstract

The present study on some wild medical taxa has been carried out from the cytological view-points by using iron-acetocarmine squashtechnique.The chromosome numbers of *Diplotaxis harra n=13, Eruca sativa n=11, both Carduus pycnocephallus n=29 and Datura stramonium n=10* were the new reports. Avariety of abnormal Chromosomal behaviours including, early & late disjunction, laggards, bridges, stickiness, and disturbed polarity were observed, albite with low frequency. All sorts of chromosomal abnormalities were counted at I &II meiotic stages. The stickiness frequency was slightly higher than others abnormalities in most stages, The highest stickiness numbers were noted in telophase I/II of *D. harra* (8.88%), *C. pycnocephalis* (4.25%), and in metaphase I/II of E. sativa (8.68%), *D. strumonium* (8.29%). The medical plant wild type used as natural genetic resources.

Keywords: cytological studies, medicinal plants, Karyotypes, chromosomal abnormality.

دراسه خلويه لسلوك الأنقسام الأختزالي غير المنتظم لبعض النباتات الطبية في منطقة الأنبار العراق

ناجي سوادي ناصر *
قسم علوم الحياة، كلية التربية للبنات، جامعة الانبار، الانبار، العراق.

الخلاصة

اجريت الدراسه الحاليه على بعض الأجناس الطبية البرية من الناحيه الخلويه، بأستخدام صبغة الكارمين الحامضية وايونات الحديد في تقانة الهرس. ان عدد المجموعة الكروموسومية لكل من نبات الخفج *Diplotaxis harra n=13* ، الجرجير *Eruca sativa n=11* ، وكلا ذيل الكلبٍ *Carduus pycnocephallus n=29* وورد البوق *Datura stramonium n=10* يعد تسجيلا جديدا، لوحظت جميع الشواذ اللكروموسومية المتضمنة الأنفصال المبكر او المتاخر، كروموسومات متاخرة، جسور كروماتينية، التصاق كروموسومي واضطرب في الأقطاب، رغم تكرارها المنخفض. جميع انواع الشدوذ الكروموسومي تم حسابها عند مراحل الأنقسام الأختزالي الأول الثاني. اظهر الألتصاق الكروموسومي تكرارا اعلى بقليل من الحلات الشاذة الأخرى لمعظم المراحل. لوحظت اعلى الأعداد للألتصاق في الطور النهائي I/II لنباتي الخفج (8.88%) وذيل الكلب (4.25%) وكذالك الطور الأستوائي I/II لنباتي الجرجير (8.68%) وورد البوق (8.29%) . انواع النباتات الطبية البرية منها تستخدم كمصادر وراثية طبيعية.

*Email: najiswady@yahoo.com

Introduction

Microsporogenesis was the developmental process leading to the production of four haploid microspores from a pollen mother cell by means of meiosis and cytokinesis. The normal course of meiosis ensured gamete viability. However, large amounts of chromosomal abnormality were detected in plant, they can cause total male sterility during the evaluation of meiotic behavior in some species [1]. In fact the primary health care of about 80% of the world's population depends on the use of medicinal plants derived from traditional medicine [2] and about 25% of conventional drugs are derived from plants that have been used traditionally.

The fact that 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 [3]. However, chromosome numbers are only known for 15-20% of angiosperm species and there is a clear correlation between chromosome number and hybrid index [4].

Diplotaxes harra L. is a desert medicinal plant, it belongs to the Brassicaceae family which is one of the largest families rich in valuable medicinal plants. It includes 338 genera and 3350 species that are distributed worldwide [5]. Thes plants were used traditionally as anti-inflammatory, anti-diabetic, anti-bacterial, anti-fungal, anti-cancer, anti-rheumatic and showed a potent insecticidal effect [5, 6]. Others reported the isolation of steroids and non-methylated fatty acids from Diplotaxes harra L. which inhibited the growth of fungi, yeast, as well as Gram-negative and Gram-positive bacteria [7].

As in other genera of Brassicaceae several gametic chromosome numbers occur in the almost completely diploid genus Diplotaxis [8], the series ranges from n=7, n=13 and the level n=8 was only recently added to the series. Also the chromosome number is n=13 were reported in Iraq [9].

Eruca sativaL., a member of the Brassicaceae family, which commonly Known as Rocket plant, or Aljarjeer was used. It is a herbaceous plant, its extract possess diversified medicinal and therapeutic properties including inhibition of tumor genesis anti-secretory, cytoprotactive, and anti-ulcer activities against experimentally-induced gastric lesions [10].E. sativa has gained greater importance as a salad vegetable and spice, especially among Middle Eastern populations and Europeans, which have a good reputation as antioxidant and anticancer against chromosomal aberration [11].

The previous report of chromosome number for E. sativa was 2n=22 from Esfahan [12]. Also the chromosome number is n=11 wase reported in Iraq [9]. Genus Carduus which belongs to the family Astraceae includes approximately 100 species worldwide [13] and is widely distributed around the Mediterranean. Seven flavonoid AL compounds, were isolated from the aerial part of Carduus pycnocephalus L. [14]. The plants of genus C. pycnocephalus are used for the treatment of various human diseases such as cold, stomach ache as well as rheumatism [15]. Genus Carduus was found to possess a wide range of biological activities such as liver tonicity, anti-inflammatory, antispasmodic, anticancer, antiviral and antibacterial [16].

The chromosome numbers of two species of genus Carduus are C. getulus 2n=26, Northern Nejev; south Beer Sheva and C. pycnocephalus 2n=60, Bet-shean vally ssw of Bet-shean [17]. Were as in our study C. pycnocephalus is n = 29, 2n = 58 as a new record in Iraq. Al-Omar [9] suggested that uncompleted chromosome numbers support the progressive evolution phenomena.

Datura stramonium L. is a widespread annual plant from the Solanaceae family. It is a wildly well-known folklore medicinal herb with both poisonous and medicinal properties and has been proven to have great pharmacological potential with a great utility and usage in medicine and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom (N-CH3) and include the anti-cholinergic drugs atropine, and scopolamine [18].

An analysis of karyotypic features is presented for five Mexican species of genus Datura consists of somatic chromosome number (2n = 24), base chromosom number x = 12 [19]. But D stramonium as in our study, n = 11. The present work may through light on possibility of using the studied medical plant species especially the wild type as natural genetic resources.

Materals and methods

For meiotic studies, young flower buds of four wild taxa species Diplotaxis harra L.and Aruca sativa L. (Brassicaceae), Carduus pycnocephallus L. (Asteraceae) and Datur stramonium L. (Solanaceae) were collected from Anbar university camp. Collection were made within March and April between 10:00 to 12:00 a.m. and fixed in freshly prepared Carnoy solution (3 parts absolute ethanol: 1 part glacial acetic acid), immediately after collection with a few drops of ferric chloride.

After 24 h in fixative, flowed by washing with distilled water 3 times, the flower buds were transferred to 70% ethanol. Meiotic cells were obtained using acetocarmine squash technique.

Result

Meiotic cells of Diplotaxis harraL. Figure-1 revealed the chromosome number as perfect bivalents 13 II , Figure-1a, at diakinesis and regular separation 13:13 Figure-1c at anaphase-I. This confirms earlier study of Al-Omar [9] on the chromosome count of D. harra as 2n = 26 and the high frequency of bivalent formation is due to the balanced number of homologous set of chromosomes favoring preferential pairing.

The frequency (%) of various types of chromosomal aberrations at different meotic stages of D. harra table-1 was observed. At metaphase I/II, Figure-1b&f exhibited bridges (3.83), stickiness (8.09) and early disjunction (2.25), while anaphase I/II, Figure-1d&g revealed laggards (4.31), stickiness (7.06) and late disjunction (5.88) and telophase I/II, Figure-1h revealed laggards (2.22), bridges (1.48), stickiness (8.88) and disturbed polarity (7.04). As a result, percentage of abnormal PMCs was generally increased gradually at metaphase, anaphase and telophase respectively, the most prominent abnormality was chromosomal stickiness in all meiotic stages, and there is no nuclei have seen.

Table.1-Frequency (%) occurrence of different meiotic chromosome configuration and behavior in Diplotaxis harra.

Meiosis Stages	Total No.of PMCs Observed	Frequency of Occurrence	Average Freaquency (%)
Diakinesis	220		
13 II			
Metaphase- I/II	470		
normal		402	85.53
Bridges		18	3.83
Stickness		38	8.09
Early Disjunction		12	2.25
Anaphase- I/II	510		
normal		422	82.75
Laggards		22	4.31
Stickness		36	7.06
Late disjunction		30	5.88
micronuclei		----	------
Telophase- I/II	540		
normal		434	8o.37
Laggards		12	2.22
Bridges		8	1.48
Stickness		48	8.88
Disturbed polarity		38	7.04

Meiotic cells of Eruca sativa Figure-2 revealed the chromosome number as perfect bivalents as 11 II, Figure-2a at diakinesis, regular separation 11:1, Figure-2d at anaphase-I and irregular separation 10:12, Figure-2f at metaphase-II. This confirm earlier study of Al-Omar on the chromosome count of E. sativa as 2n = 22 [9], and the bivalent formation to the balanced number of homologous set of pairing.

The frequency (%) of various types of chromosomal aberrations at different meotic stages of E. sativ table-2 was observed. At metaphase I/II, Figure-2b&c, exhibited bridges (4.15), stickiness (8.68)

and early disjunction (4.72), while anaphase I/II, Figure-2d&g revealed laggards (4.60), stickiness (5.23), late disjunction (3.77) and telophase I/II, Figure-2e&h revealed laggards (3.33), bridges (2.22), slickness (5.19) and disturbed polarity (1.48).). As a result, percentage of abnormal PMCs was generally higher at metaphase (especially stickiness) than anaphase and telophase.

Meiotic cells of Carduus pycnocephalis Figure-3 revealed the chromosome number as perfect bivalents as 29 II, Figure-3a&b at diakinesis, this confirm as new record in Iraq, but in Palestine the same species 2n = 60 [17].

Table.2-Frequency (%) occurrence of different meiotic chromosome configuration and behavior in Eruca sativa.

Meiosis Stages	**Total No.of PMCs Observed**	**Frequency of Occurrence**	**Average Freaquency (%)**
Diakinesis	180		
11 II			
Metaphase- I/II	530		
normal		437	82.45
Bridges		22	4.15
Stickness		46	8.68
Early Disjunction		25	4.72
Anaphase- I/II	478		
Normal		413	86.40
Laggards		22	4.60
Stickness		25	5.23
Late disjunction		18	3.77
micronuclei		-----	------
Telophase- I/II	540		
normal		474	87.78
Laggards		18	3.33
Bridges		12	2.22
Stickness		28	5.19
Disturbed polarity		8	1.48

The frequency (%) of various types of chromosomal aberrations at different meotic stages of C. pycnocephalis table-3 was observed. At metaphase I/II, Figure-3e, exhibited bridges (2.78), sickness (3.89) and early disjunction (3.33), while anaphase I/II (Fig.3c&f) revealed laggards (4.72), stickiness (3.94), late disjunction (3.94) and micronuclei (3.54) and telophase I/II, Figure-3d,g&h revealed laggards (3.97), bridges (0.85), stickiness (4.25) and disturbed polarity (3.12). As a result percentage of abnormal PMCs was generally higher at anaphase than metaphase and telophase and macronuclei have seen at diakinesis and anaphase-I.

Table.3-Frequency (%) occurrence of different meiotic chromosome configuration and behavior in Carduus pycnocephalis.

Meiosis Stages	**Total No.of PMCs Observed**	**Frequency of Occurrence**	**Average Freaquency (%)**
Diakinesis	92		
29 II			
Metaphase- I/II	360		
normal		324	90.0
Bridges		10	2.78
Stickness		14	3.89
Early Disjunction		12	3.33
Anaphase- I/II	254		
normal		213	83.86
Laggards		12	4.72
Stickness		10	3.94
Late disjunction		10	3.94
micronuclei		9	3.54
Telophase- I/II	353		
normal		310	87.82
Laggards		14	3.97
Bridges		3	0.85
Stickness		15	4.25
Disturbed polarity		11	3.12

Meiotic cells of Datura strumonium Figure-4 revealed the chromosome number as perfect bivalents as 11 II, Figure-4a at diakinesis, rregular separation 10:10, Figure-4c at anaphase-I. The base chromosom number of D stramonium X = 12 [19]. The new result mightbe n= 12-1 duto unstable chromosome numbers.

The frequency (%) of various types of chromosomal aberrations at different meotic stages of D strumonium table-4 was observed. At metaphase I/II, Figure-4b&e, exhibited bridges (3.55), stickiness (8.29) and early disjunction (2.84), while anaphase I/II, Figure-4c&f revealed laggards (4.39), stickiness (5.37) and late disjunction (4.88) and telophase I/II, Figure-4d&g revealed laggards (4.61), bridges (4.88), stickiness (6.50) and disturbed polarity (5.42). As a result, percentage of abnormal PMCs was generally higher at metaphase than anaphase and telophase.

Table 4-Frequency (%) occurrence of different meiotic chromosome configuration and behavior in Datura strumonium.

Meiosis Stages	**Total No.of PMCs Observed**	**Frequency of Occurrence**	**Average Freaquency (%)**
Diakinesis	132		
11 II			
Metaphase- I/II	422		
normal		360	85.31
Bridges		15	3.55
Stickness		35	8.29
Early Disjunction		12	2.84
Anaphase- I/II	410		
normal		350	85.37
Laggards		18	4.39
Stickness		22	5.37
Late disjunction		20	4.88
micronuclei		----	------
Telophase- I/II	369		
normal		290	78.59
Laggards		17	4.61
Bridges		18	4.88
Stickness		24	6.50
Disturbed polarity		20	5.42

Discussion

During the present investigation, the meiotic division study of four medicenal plants,(Diplotaxis harra, Aruca sativa, Carduus pycnocephallus and Datur stramonium) were exhibited karyotypes and meiotic abnormalities in most meiotic stages, generally the highest numbers of abnormality were noted at metaphase of E. sativa , at anaphase of C. pycnocephallus, at telophase of both D. harra and D. stramonium. The results also showed the spectrum of chromosomal abnormalities, included a ridges, laggards, stickiness, early disjunction and disturbed polarity, a comparatively higher proportion of stickiness in most stages.

These variable results might be due to the variety of plant species which have different genetic resources of ability to resist environmental factors effects.

Early disjunction of chromosomes movement was observed at metaphase I and II stages. This precocious movement of chromosomes might have occurred due to disturbed homology for chromosome pairing, disturbed spindle mechanism or inactivation of spindle mechanism [20]. Along with the precocious separation of univalent, the bivalents were also observed to move ahead and seemed as stray chromosome, this may move to one pole. Irregular chromosome segregation in meiosis I and II resulting into unequal distribution of chromosome or loss of a complete bivalent at later stage could be the result of the non-oriented bivalents formed due to spindle dysfunctioning or they could be due to the formation of univalent at diakinesis or metaphase I, which shows an inability to congregate on the equatorial plate resulting in the formation of micronuclei and abnormal pollen grains [21]**.**

The occurrence of laggards in the present study were presented in all stages has also been reported previously by many workers such as [22, 23]. Delayed terminalisation and/or failure of chromosomal movement, following spindle fiber discrepancies have led to lagging chromosomes. The fragments which appeared on the breakage of bridges, as a result of spindle fibers functioning to pull the

chromosome toward poles, formed laggards [24].Stickiness was observed as a common meiotic abnormality among the chromosomes occurred in all metaphase I/II, anaphase I/II and telophase I/II meiotic stages. Stickiness has also been reported to occur spontaneously due to known and unknown environmental factors in various plants [25]. According to [26] stickiness appears as a result of disturbances in the nucleic acid metabolism in the cell. It may also cause movement of whole bivalent towards one pole at anaphase due to the non-disjunction of homologous chromosomes.

Bridges occurred both at anaphase I &II and metaphase I & II stages. Bridge might have occurred as a result of delayed terminalization, stickiness of chromosome ends, failed of chromosome movement [27] lead to unequal separation of chromosomes [22]. The laggards and non-orientedchromosomes when fail to reach the poles in time to be included in the main telophase, form micro nuclei leading to multinucleate condition[28]. The disturbed polarity at telophase stage could be due to spindle disturbances.

The indication of cytological disturbances as an equele chromosome numbers in Datura Figure-4c and aberration in the meiotic cells division is of great value, as it results in genetic damage that is handed over to next generation [29]. These phenomina may be attributed to the nature and potency of mutagen and to the underlying factors such as complex structural changes or to the nature of the genes responsible for meiotic divisions. Hence, the conservation of such medically important plants is the requirement of the time. However most of the plants cannot leave their growing sites and thus, may become victims of ecological disturbances directly due to pollution. The cytogenetic researches are very important as the basis of means for maintaining a stable ecosystem throughout the entire biological kingdom.

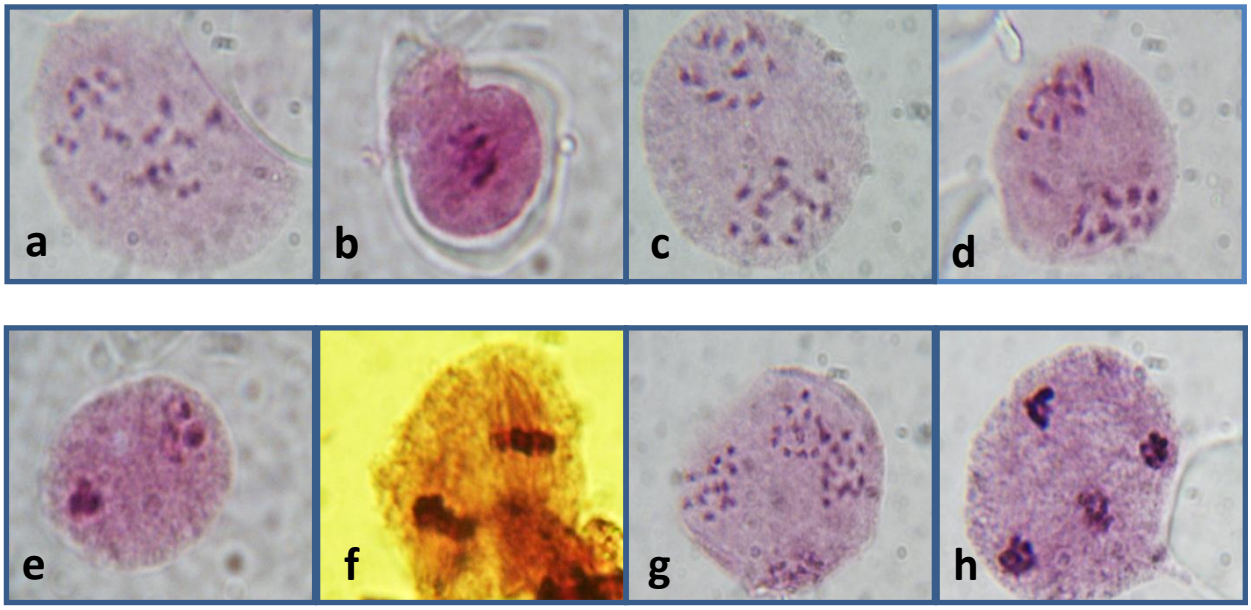

Figure 1-Photomicrographs (400X) of Meiosis I &II in Diplotaxis harra. (a) Normal Diakinesis (n=13), (b) Sticky 3 grouped chromosomes at metaphase-I (c) Regular separation (13:13), (d) laggard & stickiness at anaphase-1; (e) Sticky chromosomes at telophase-I; (f) Bridges & stickness at metaphase-II; (g) Late disjunction & at anaphase-II; (h) Stickiness & disturbed polarity at telophase-II.

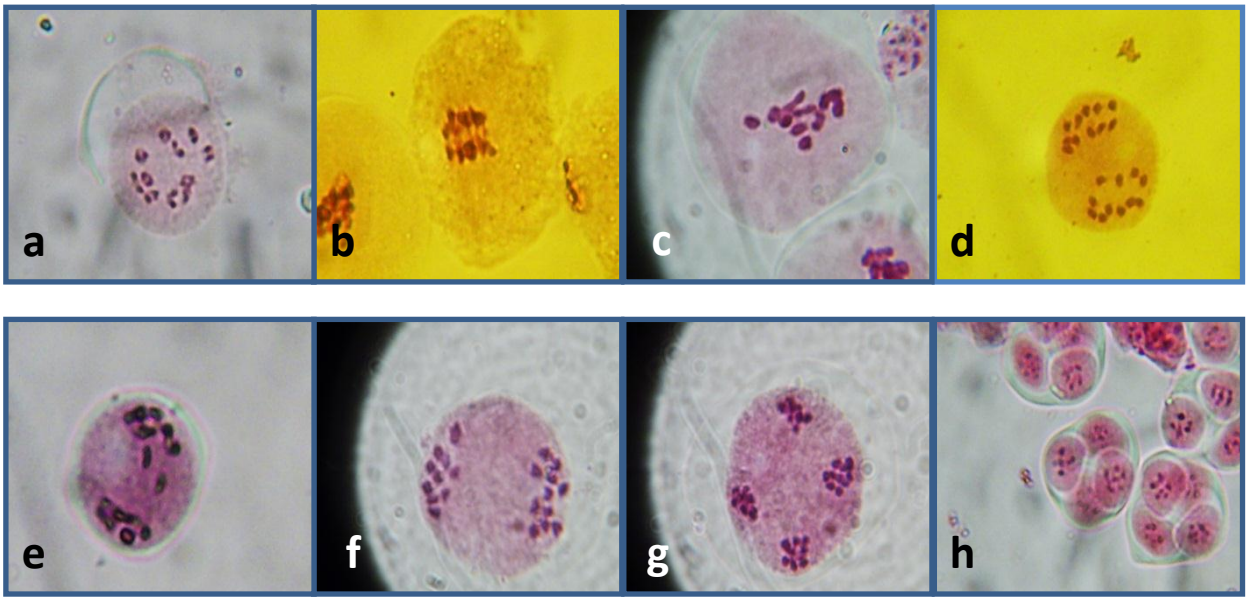

Figure 2-Photomicrographs (400X) of Meiosis I &II in Eruca sativa. (a) Diakinesis (n=11); (b) Bridges, early disjunction & (c) stickiness at metaphase I; (d) Regular separation (11:11) at anaphase I; (e) Laggard &stickiness at telophase-1; (f) Irregular division (10:12) at metaphase-II; (g) Laggard &stickiness at anaphase-II; (h) Tetrad at telophase-II.

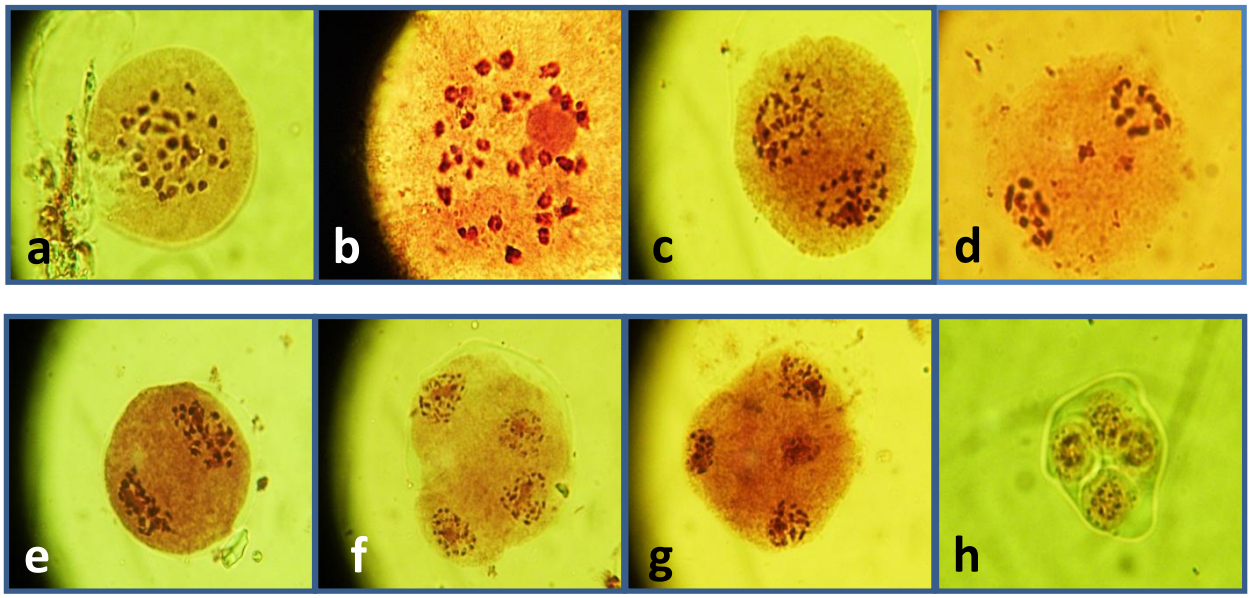

Figure 3- Photomicrographs (400X) of Meiosis I &II in Carduus pycnocephalus . (a &b) Normal &macronuclei Diakinesis (n=29); (c)Macronuclei at anaphase-I; (d) Laggards &stickiness at telophase-1; (e) Stickiness & early disjunction at metaphase-II; (f) Late disjunction at anaphase-II; (g)Laggards, disturbed polarity &(h) irregular Tetrad at telophase-II.

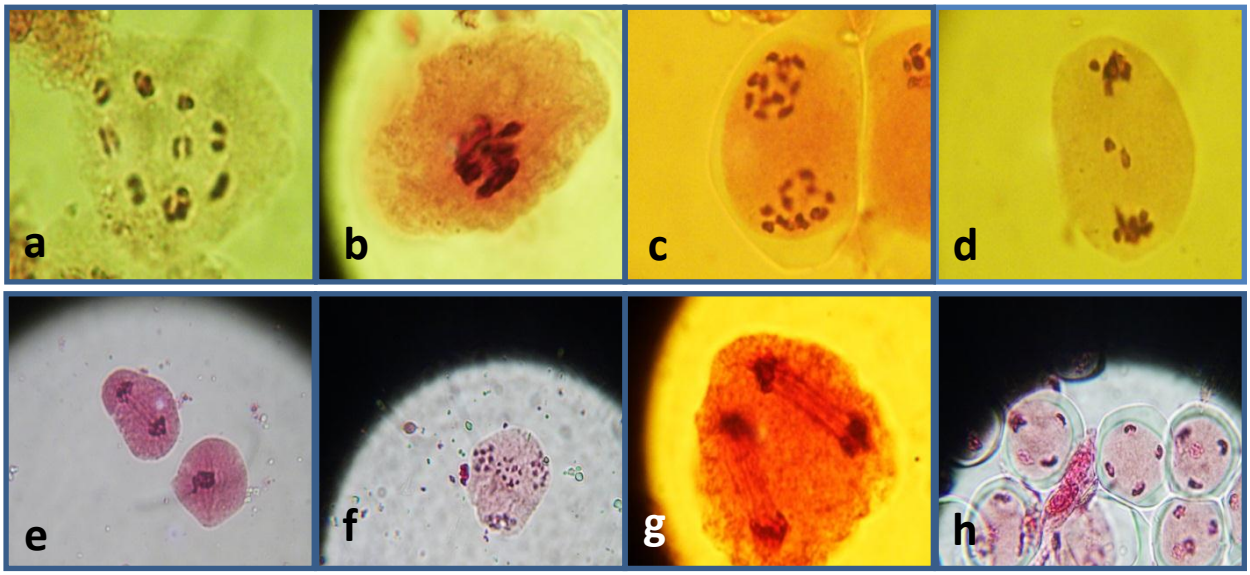

Figure 4-Photomicrographs (400X) of Meiosis I &II in Datura stramonium. (a) Normal Diakinesis with 10 bivalents (n=10); (b)Intense chromosomes stickiness at metaphase-I; (c) Regular separation (10:10)at anaphase I; (d) Laggard & stickiness at telophase-1; (e) Bridges & stickiness at metaphase-II; (f) Late disjunction at anaphase-II; (g) Bridges & stickiness &(h) Cytomexis at telophase-II.

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