*Kuwait J. Sci. Eng.* **30**(2) pp. 67-80, 2003

# A cytogenetic investigation of some wild species from Kuwaiti flora, IV

GHANIMA A. MALALLAH, MODI AL-DOSERI,
TALAAT ATTIA AND SMITHA PARIYANI

*Department of Biological Sciences, Botany Program, Kuwait University*
*P.O.Box 5969, Safat 13060, Kuwait. e-mail: ghanima@kuc01.kuniv.edu.kw*

## ABSTRACT

The chromosome numbers of 21 wild species of flowering plants are determined. Chromosome counts reported for the first time are *Anchusa hispida*, 2n = 20 and n = 10; *Farsetia aegyptia*, 2n = c.60 and n = 30; *Frankenia pulverulenta*, 2n = 30; *Haplophyllum tuberculatum*, 2n = 18 and n = 9; *Neurada procumbens*, n = 7; *Oligomeris subulata*, 2n = 30 and n = 15; *Roemeria hybrida*, 2n = 20 and n = 10; *Rostraria pumila*, n = 6, *Savignya parviflora*, 2n = 30; *Silene villosa*, n = 12.

**Keywords:** Chromosome number; meiosis; mitosis; Kuwaiti flora; wild plants

## INTRODUCTION

There is a lack of information regarding the chromosome number of wild species from Kuwaiti flora. Therefore, a plan of work was initiated to cover some aspects of the cytology of wild species of the Kuwaiti flora. Only recently reports have appeared on the chromosome number, chromosome behavior during mitosis and meiosis, and karyotyping (Malallah & Brown 1999, Malallah *et al*. 2001 (a, b, c)) of Kuwaiti flora.

Cytogenetic studies, which include chromosome number, structure and behavior, can profice basic and valuable information for the identification of a species for nature conservation and other applied programs as well as for taxonomic, genetic and molecular studies. This kind of work is needed as the natural vegetation is threatened by shortage of rain, grazing and human activities.

The present work covers 21 wild species of flowering plants from different families of the Kuwaiti desert.

## MATERIALS AND METHODS

Seeds and buds were collected from, at least, three different plants growing at the same site from various localities in Kuwait. The seeds were germinated on

moist filter papers contained in Petri dishes in a controlled growth chamber. The seed samples were initially maintained at 16°C for 12 hours in the dark, subsequently at 23°C for 12 hours in the light in a 24-hour dark/light cycle. Actively growing root tips were excised and pretreated in 0.05% colchicine for 4 hours in the dark in order to have C-metaphases, and then fixed in Carnoy's fixative (1:3 glacial acetic acid: ethanol) until use. For slide preparation, the excised root tips were washed with distilled water for 4-5 minutes, hydrolyzed in 1 N hydrochloric acid for 10-12 minutes at 60°C before finally rinsing in distilled water and squashing in acetocarmine stain.

Young flower buds were collected and fixed in Carnoy's fixative until further use. The anthers were squashed and stained in a drop of 1-% acetocarmine and covered with a clean cover glass. Microscopic analyses were carried out with an Olympus BH-2 photomicroscope and the micrographs were taken using the 100x oil immersion lens on temporary or permanent preparations.

The nomenclature and synonyms are in accordance with the latest checklist of the Kuwaiti flora (Boulos & Al-Doseri 1994). voucher specimens of the species are deposited in a herbarium (KTUH) and in our laboratory.

## RESULTS AND DISCUSSION

In Kuwait, the microspores of many wild plant species mature at unpredictable times during the short growing season, leading to a considerable difficulty in getting cells at suitable stages for cytogenetic analyses. Usually, the amounts of collected seeds were very low due to the wind, which readily sweeps away most of the mature seeds. Intentionally, not all the mature seeds are collected, for the purpose of seed conservation in the environment. Preliminary studies showed that the viability of some of the collected seed was rather low (data not presented) and when germinated yielded low numbers of roots. However, sufficient numbers of cells with well spread chromosomes were observed and analyzed to verify each count.

The families, genera and species and the available data for somatic and gametic numbers with map references are listed below in alphabetical order in Table 1. The characteristic observations are followed with literature data for each corresponding species. The analysis of chromosome behavior during mitosis or meiosis was done along with chromosome counting and any differences from normal were noted.

Most of the literature data were accumulated from published data documented in indices of plant chromosome numbers (IPCN), therefore, to minimize long lists of references related to each species, we will refer to the original indices that include those references.

**Table 1.** The diploid and haploid numbers of representatives of wild plant species from Kuwaiti flora with voucher number, site and date of collection. (* for new count)

| Sr. # | Taxon | Voucher No:, Site of Collection & Date | GPS Location | n | 2n |
|---|---|---|---|---|---|
| 1 | **Brassicaceae**<br>*Carrichtera annua* (L.) DC. | 2000MM238<br>Al-Salmi, 13.1.2001 | 29°06'N<br>46°51'E | - | 16 |
| 2 | *Diplotaxis harra* (Forssk.) Boiss. | 2000MM143<br>Al-Salmi, 17.1.2000 | 29°06'N<br>47°39'E | 12, 13 | 24, 26 |
| 3 | *Farsetia aegyptia* Turra | KTM 4723<br>Subiya, 2.3.2000 | 29°35'N<br>48°10'E | 30* | c.60* |
| 4 | *Savignya parviflora* (Delile) Webb | 2000 MM 156<br>Sulaibiya KISR, 21.2.2000 | 29°09'N<br>47°41'E | 15 | 30* |
| 5 | **Boraginaceae**<br>*Anchusa hispida* Forssk. | 2001MM271<br>Mina Al-Zoor, 21.1.2001 | 28°44'N<br>48°21'E | 10* | 20* |
| 6 | *Arnebia decumbens* (Vent.) Coss. & Kral. | Modi Al-Doseri4774<br>Um Neqqa, 20.3.2000 | 30°05'N<br>47°43'E | 11 | 22 |
| 7 | **Caryophyllaceae**<br>*Silene villosa* Forssk. | 2001MM314<br>Sulaibiya KISR, 31.1.2001 | 29°09'N<br>47°41'E | 12* | - |
| 8 | *Spergularia diandra* (Guss.) Heldr. & Sart. | 2001MM314<br>Salmiya KISR<br>28.1.2001 | 29°17'N<br>47°48'E | 9 | 18 |
| 9 | *Spergularia marina* (L.) Griseb. | 2000MM166<br>Wafra, 24.2.2000 | 28°33'N<br>48°04'E | - | 36 |
| 10 | **Cistaceae**<br>*Helianthemum lippii* (L.) Dum. Cours. | 2000MM180<br>Failka, 2.3.2000 | 29°26'N<br>48°18'E | 10 | 20 |
| 11 | **Frankeniaceae**<br>*Frankenia pulverulenta* L. | 2000MM168<br>Wafra, 24.2.2000 | 28°33'N<br>48°04'E | - | 30* |
| 12 | **Malvaceae**<br>*Malva parviflora* L. | 2000MM196<br>Um Al Maradam, 20.3.2000 | 28°40'N<br>48°38'E | - | 42 |
| 13 | **Neuradaceae**<br>*Neurada procumbens* L. | 2001 MM 224<br>Khiran, 6.1.2001 | 28°40'N<br>48°23'E | 7* | 14 |
| 14 | **Papaveraceae**<br>*Roemeria hybrida* (L.) DC. | 2000MM172<br>Um Al Rimam, 28.2.2000 | 29°30'N<br>47°45'E | 10* | 20* |

**Table 1. (cont'd)** The diploid and haploid numbers of representatives of wild plant species from Kuwaiti flora with voucher number, site and date of collection. (* for new count)

| Sr. # | Taxon | Voucher No:, Site of Collection & Date | GPS Location | n | 2n |
|---|---|---|---|---|---|
| 15 | **Poaceae**<br>*Bromus madritensis* L. | 2002 SP 366<br>Khiran, 27.6.2002 | 28°40'N<br>48°38'E | 14 | 28 |
| 16 | *Bromus tectorum* L. | 2000MM120<br>King Fahad Road, 15.1.2000 | 29°03'N<br>48°03'E | - | 14 |
| 17 | *Lolium rigidum* Gaudin | 2000MM118<br>King Fahad Road, 15.1.2000 | 29°03'N<br>48°03'E | 7 | 14 |
| 18 | *Phalaris minor* Retz. | 1999MM107<br>Al-Salmi, 14.3.1999 | 29°06'N<br>47°39'E | 14 | - |
| 19 | *Rostraria pumila* (Desf.) Tzvelev | Mary Leo 124<br>Al-Salmi, 19.3.1998 | 29°06'N<br>46°41'E | 6* | 12 |
| 20 | **Resedaceae**<br>*Oligomeris subulata* (Webb & Berth.) Webb | 2000MM184<br>Failaka, 2.3.2000 | 29°26'N<br>48°18'E | 15* | 30* |
| 21 | **Rutaceae**<br>*Haplophyllum tuberculatum* (Forssk.) | 2002 SP 372<br>Khiran, 26.7.2002 | 28°40'N<br>48°23'E | 9* | 18* |

## Brassicaceae

***Carrichtera annua* (L.) DC.** Fig.1 (A)

2n = 16 n = ?

The genus *Carrichtera* showed the basic number of x = 8 (Darlington & Wylie 1955). The diploid number of this species in Kuwait confirms the previous chromosome number reports (Darlington & Wylie 1955, Bolkhovskikh *et al.* 1969, Moore 1973, 1977, Goldblatt 1981, 1984, 1985, Goldblatt & Johnson 1994, 1996, 1998) from different parts of the world. All mitotic chromosomes were medium sized and metacentric, except one acrocentric pair.

***Diplotaxis harra* (Forssk.) Boiss.** Fig.1 ($B_1$ & $B_2$)

2n = 24, 26 n = 12, 13

Several microsporocytes from anthers of a bud showed the diploid number of 24 (Fig. $1B_1$) with microspores showing 12 chromosomes, while other cells showed the diploid number of 26 (Fig. $1B_2$) with microspores showing 13 chromosomes. The genus *Diplotaxis* was found by Darlington & Wylie (1955) to have a basic number of x = 7, 9 & 11. Previously reported data (Bolkhovskikh *et al*. 1969) demonstrated the inclusion of two B chromosomes in cells of two other species of the genus *Diplotaxis*. However, three studies showed that *D. harra* contains n = 13 (Moore 1974, Goldblatt 1985, Goldblatt & Johnson 1990), and only one study showed 2n = 26 (Goldblatt 1985) which is confirmed by one of our values. Another study showed 2n = 38 (Goldblatt 1988) for the same species. The difference between the values may be due to the presence of undetermined number of B chromosomes in cells of some anthers or other unknown reasons. An interesting observation was recorded related to the presence of cytoplasmic channels connecting two or three cells. A few channels were observed to contain one or two chromosomes. This finding will be the subject of a separate study.

***Farsetia aegyptia* Turra.** Fig.1 (C) and Fig. 3 (A)

2n = c. 60 n = 30

Other counts of n = 24 (Glodblatt 1985) and of 2n = c. 72 (Bolkhovskikh *et al*. 1969, Goldblatt 1981, 1984, Goldblatt & Johnson 1990) have also been reported.

***Savignya parviflora* (Delile) Webb** Fig.1 (D)

2n = 30 n = 15

The gametic number is consistent with the only three studies found in the literature (Goldblatt 1981, 1985). The chromosomes were found to be so small that it was very difficult to make further observations.

## Boraginaceae

***Anchusa hispida* Forssk.** Fig.1 (E)

2n = 20 n = 10

The genus *Anchusa* was shown to have variable values of basic number such as 6, 8, 9, and 11 (Darlington & Wylie 1955). The indices of plant chromosome numbers indicate many species of the genus show a gametic number of 8, 8 + B and 16, while the somatic number was mostly of 16 or 32. We could not observe any B chromosomes in our samples. The values in our work were different and

might indicate the presence of another basic number either as 5 or 10. Although the mitotic preparations did not allow karyotyping of chromosomes, the cells showed chromosomes of large sizes compared with other Kuwaiti plants examined.

***Arnebia decumbens* (Vent.) Coss. & Kral.** Fig.1 (F)

2n = 22 n = 11

The few previous reports for this taxon were 2n = 8, 22 (Bolkhovskikh *et al.* 1969, Goldblatt 1981) and n = 11 (Goldblatt 1981, Goldblatt & Johnson 2000).

No basic chromosome number has been previously published for *Arnebia* but most other species of this genus have chromosome numbers of 2n = 14 (Moore 1997, Goldblatt & Johnson 1990, 1996), suggesting that x = 7.

## Caryophyllaceae

***Silene villosa* Forssk.** Fig.1 (G)

2n = 24 n = 12

The gametic number presented here is the first to be reported. The diploid number here confirmed the sole previous report (Goldblatt & Johnson 1994). The chromosomes were of small size and metacentric.

***Spergularia diandra* (Guss.) Heldr. & Sart.** Fig.1 (H)

2n = 18 n = 9

The somatic number of chromosomes is consistent with other values from other parts of the world. The gametic number of this species found here is the second to be reported and confirmed the first value (Goldblatt 1981). Fig. 1H shows 9 bivalents.

**Spergularia marina (L.) Griseb.**

2n = 36 n = ?

The diploid number of 36 is consistent with the number from other parts of the world (Bolkhovskikh *et al.* 1969, Moore 1971, 1973, Goldblatt 1988, Goldblatt & Johnson 1990, 1996). Several reports showed the value of 2n = 18 and n = 18 within this species (Moore 1971, 1973, Goldblatt 1981). This species could be a tetraploid on the basis of x = 9 (Darlington & Wylie 1955), but because of the very small size of the chromosomes they were difficult to karyotype.

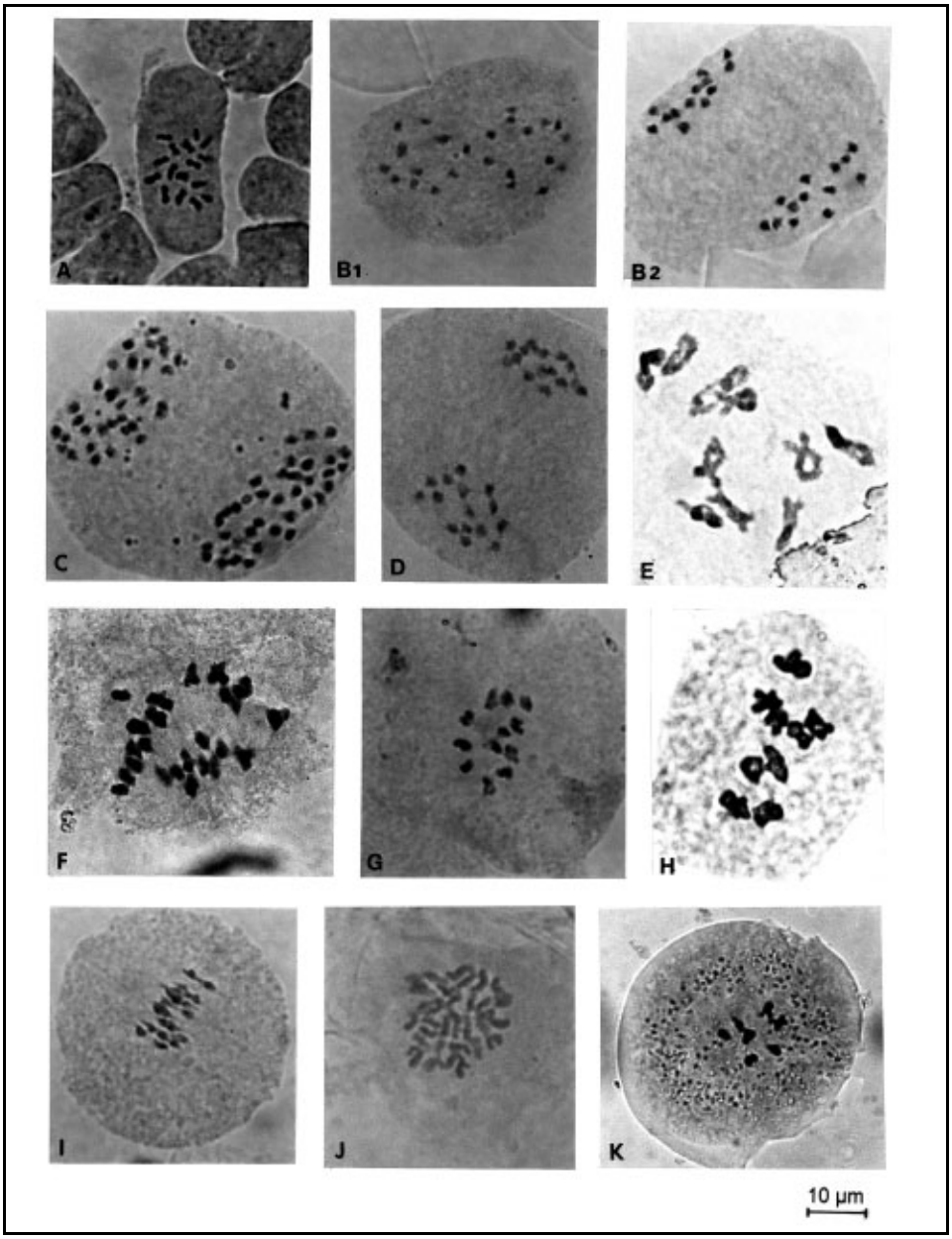


**Fig.1.** Photomicrographs of chromosomes of 11 wild plant species from Kuwaiti flora.

*A*. *Carrichtera annua* 2n = 16 (metaphase-mitosis), $B_1$. *Diplotaxis harra* 2n = 24 (anaphase I), $B_2$. *Diplotaxis harra* 2n = 26 (anaphase I), C. *Farsetia aegyptia* n = 30 (anaphase I), D. *Savignya parviflora* n = 15 (anaphase 1), E. *Anchusa hispida* n = 10 (diakinesis), F. *Arnebia decumbens* 2n = 22 (metaphase I), G. *Silene villosa* n = 12 (telophase I), H. *Spergularia diandra* n = 9 (metaphase I), I. *Helianthemum lippii* 2n = 20 (metaphase I), J. *Frankenia pulverulenta* 2n = 30 (metaphase-mitosis), K. *Neurada procumbens* n = 7 (microspore).

## Cistaceae

***Helianthemum lippii* (L.) Dum. Cours.** Fig.1 (I) and Fig.3 (B)

2n = 20 n = 10

The somatic and gametic number of chromosomes is consistent with previously reported values (Bolkhovskikh *et al*. 1969, Moore 1973, Goldblatt & Johnson 1990, 1991, 1996). This is the third report confirming the gametic number of 10. No cytokinesis was observed either in telophase I or in telophase II.

## Frankeniaceae

***Frankenia pulverulenta* L.** Fig.1 (J)

2n = 30 n = ?

This species which is the only species from this family found in Kuwait. This diploid number shows the possible hexaploid nature (2n = 6x = 30) of this species since Darlington & Wylie (1955) mentioned the possibility of x = 5. Several reports (Darlington & Wylie 1955, Bolkhovskikh *et al*. 1969, Moore 1977, Goldblatt 1984) showed that 2n = 20 (2n = 4x = 20). However, *F. aucheri* (Moore 1972, 1973) and *F. laevis* (Goldblatt 1994, 1998) were found with 2n = 30.

## Malvaceae

***Malva parviflora* L.**

2n = 42 n = ?

The diploid number is consistent with other values from different parts of the world (Darlington & Wylie 1955, Bolkhovskikh *et al*. 1969, Moore 1977, Goldblatt 1981, 1984, Goldblatt & Johnson 1990, 1998, 2000). The chromosomes were small and most of them were metacentric.

## Neuradaceae

***Neurada procumbens* L.** Fig.1 (K)

2n = 14 n = 7

Darlington & Wylie (1955) and Bolkhovskikh *et al*. (1969) reported the somatic chromosome number of 2n = 12, 14 for this species. The gametic number in this study is the first to be reported. The A I chromosomes were of short length showing centric or acrocentric centromeres. No cytokinesis was observed during telophase I.

## Papaveraceae

***Roemeria hybrida* (L.) DC** Fig.2 ($A_1$ & $A_2$)

2n = 20 n = 10

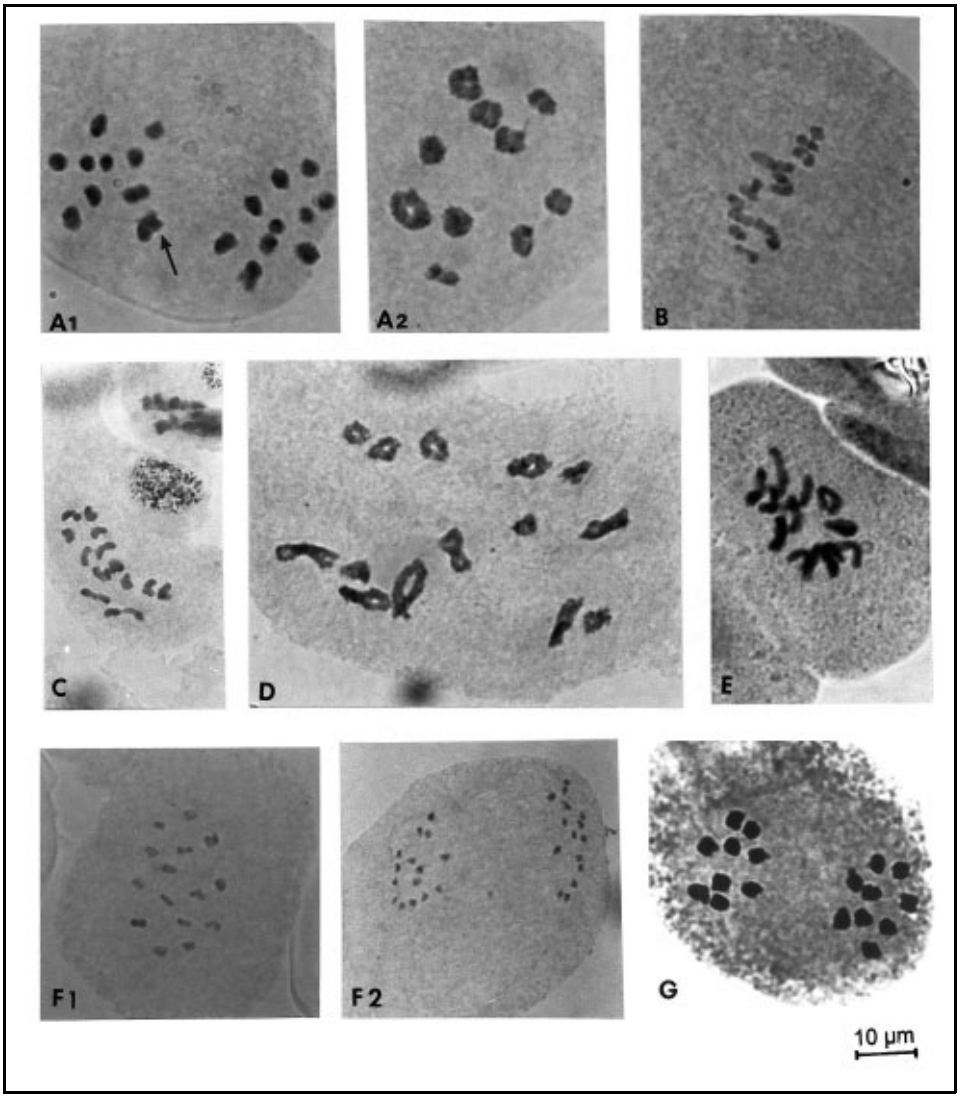


**Fig.2.** Photomicrographs of chromosomes of another 10 species of wild plants from Kuwaiti flora.

$A_1$. *Roemeria hybrida* 2n = 20 (anaphase I), $A_2$. *Roemeria hybrida* n = 10 (late diakinesis), B. *Bromus madritensis* n = 14 (metaphase I), C. *Lolium rigidum* 2n = 14 (metaphase-mitosis), D. *Phalaris minor* n = 14 (diakinesis), E. *Rostraria pumila* 2n = 12 (metaphase-mitosis), $F_1$. *Oligomeris subulata* n = 15 bivalents (metaphase I), $F_2$. *Oligomeris subulata* 2n = 30 (anaphase I). G. *Haplophyllum tuberculatum* n = 9 (anaphase 1).

Previously reported somatic chromosome numbers for this species are 2n = 22 (Darlington & Wylie 1955, Bolkhovskikh *et al*. 1969, Goldblatt & Johnson 1990, 2000), 2n = 24 (Goldblatt & Johnson 1990, 1994) and 2n = 16 (Goldblatt & Johnson 1996). The above somatic and gametic numbers of chromosomes are the first to be reported for this species. The pollen mother cells showed ten bivalents, each with at least one chiasma. The migration of the members of the largest bivalent occurred late during anaphase I as shown in Fig.2 ($A_1$)-arrow.

## Poaceae

***Bromus madritensis* L.** Fig.2 (B) and Fig.3 (C)

2n = 28 n = 14

The somatic and gametic numbers were found to be consistent with many previous reports from other parts of the world (Moore 1971, 1972, 1973, 1977, Goldblatt 1981, Goldblatt & Johnson 1990, 1991).

***Bromus tectorum* L.**

2n = 14 n = ?

The somatic number of chromosomes of this species is consistent with many others reports from different parts of the world (Darlington & Wylie 1955, Bolkhovskikh *et al*. 1969, Moore 1970, 1972, 1973, 1977, Goldblatt 1981, 1984, 1988, Goldblatt & Johnson 1994, 2000).

***Lolium rigidum* Gaudin** Fig.2 (C)

2n = 14 n = 7

The somatic and gametic chromosome numbers are consistent with the values from other parts of the world (Darlington & Wylie 1955, Ornduff 1968, Bolkhovskikh *et al*. 1969, Moore 1972, 1973, 1974, 1977, Goldblatt 1981, 1984, Goldblatt & Johnson 1996, 1998).

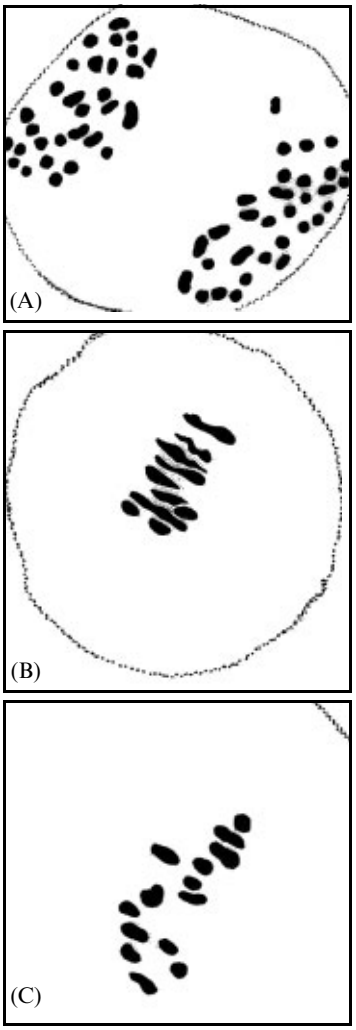


**Fig.3.** Interpretive drawings for three micrographs from Figs. 1&2. A = Fig.1 (C), B = Fig.1 (I), C = Fig.2 (B)

***Phalaris minor* Retz.** Fig.2 (D)

2n = ? n = 14

The gametic number was found to be consistent with other values from other parts of the world (Moore 1972, 1973, Goldblatt 1981, 1984, 1985, Goldblatt & Johnson 1990, 1994, 2000).

***Rostraria pumila* (Desf.) Tzvelev** Fig.2 (E)

Syn. *Lophochloa pumila* (Desf.) Bor.

2n = 12 n = 6

The previously reported values were for the synonym as n = 7, 13 (Goldblatt 1988, Goldblatt & Johnson 2000) and 2n = 12 (Goldblatt & Johnson 1990). Another report showed the gametic number of *Rostraria pumila* is n = 7 (Goldblatt & Johnson 1990). The differences in values may be due to uncertainty of the chromosome counts or taxonomic confusion.

## Resedaceae

***Oligomeris subulata* (Webb & Berth.) Webb.** Fig.2 ($F_1$ & $F_2$)

2n = 30 n = 15

The somatic and the gametic number of chromosomes are being reported for the first time. Fig.2 $F_1$ shows a microspore with 15 small chromosomes.

## Rutaceae

***Haplophyllum tuberculatum* (Forssk.)** Fig.2 (G)

2n = 18 n = 9

There is no cytological data regarding this species whatsoever. Another species of the genus *Haplophyllum* was found to have n = 9 and 2n = 18 (Moore 1974, 1977, Goldblatt & Johnson 1990, 1991, 1994, 1996). Most of the chromosomes were metacentric.

## ACKNOWLEDGEMENTS

We thank the Research Administration, Kuwait University, for the financial support of project number SO 078.

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***Submitted :*** *4/3/2003*
***Revised :*** *2/7/2003*
***Accepted :*** *12/7/2003*

# دراسة وراثة خلوية لبعض نباتات الكويت البرية (4)

**غنيمة مال الله و موضي الدوسري و طلعت عطية و سميثه برياني**

قسم العلوم البيولوجية – برنامج النبات – كلية العلوم – جامعة الكويت
ص.ب. 5969، الصفاة 13060، الكويت

## خلاصة

تهدف هذه الدراسة إلى تحديد العدد الكروموسومي لـ 21 نوعاً من النباتات الزهرية البرية البرية. وقد تم في هذه الدراسة تسجيل أرقام صبغية لأول مرة هي:

2n = 20, n = 10 *Anchusa hispida*

2n = c.60, n = 30 *Farsetia aegyptia*

2n = 30 *Frankenia pulverulenta*

2n = 18, n = 9 *Haplophyllum tuberculatum*

n = 7 *Neurada procumbens*

n = 12 *Silene villosa*

2n = 30 *Savignya parviflora*

n = 6 *Rostraria pumila*

2n = 20, n = 10 *Roemeria hybrida*

2n = 30, n = 15 *Oligomeris subulata*