



## A Cytotaxonomic study on *Minuartia* L. (Caryophyllaceae) in Iraq

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**Abstract:** Chromosome count, karyotypic character analysis, meiotic studies, monoploid karyograms and ideograms were performed in six taxa of *Minuartia* growing in Iraq (*M. hamata*, *M. hybrida* subsp. *hybrida*, *M. intermedia*, *M. meyeri*, *M. picta* and *M. hybrida* subsp. *turcica*). Species of *M. hamata* and *M. meyeri* showed  $2n=2x=30$  chromosome number, while *M. hybrida* subsp. *hybrida* and *M. intermedia* were diploid (26). The chromosome number ( $n=x$ ) of six species was studied, and was found to be  $n=15$  in *M. hamata* and *M. meyeri*, 13 in *M. hybrida* and *M. intermedia*, while in *M. picta* we recorded values of  $n=11$  and 14. Karyotype analysis of this species was first carried out in our study. Analysis of metaphases showed that the karyotype formula was mainly metacentric, submetacentric, and sub acrocentric. The sizes of the chromosomes were mainly small and very small. The course of meiosis varied from normal to abnormal. Abnormal microsporogenesis formation of two bridge chromosomes was detected in *M. hamata* and one bridge chromosome in *M. intermedia* and *M. meyeri*. Formation of laggard's chromosomes was detected in *M. hamata*, *M. meyeri* and *M. intermedia*. As well as ring chromosome was showed in *M. hybrida* subsp. *hybrida*, also, some cells contain triad cell in metaphase stage instead four cells, as well as founded cell, contains two nuclei in same species which led to reduced pollen fertility and differences in pollen grain size.

**Keywords:** *Minuartia*, Caryophyllaceae, Chromosome, Karyotype analysis.

### Introduction

The genus *Minuartia* L. is placed in the subfamily Alsinoideae (DC.) Fenzl of the Caryophyllaceae, which contains about 175 species found in the temperate to arctic - alpine regions of Northern Hemisphere (Bittrich, 1993; Nicola & Pozner, 2013). In Iraq, nine species from *Minuartia* have been described. Several studies have reported different chromosome counts in the *Minuartia*

the most frequent chromosome number in the *Minuartia* is  $2n = 2x = 30$ . The diploid chromosome number of *M. verna*, *M. stricta*, *M. rubella* and *M. laricifolia* was reported as  $2n = 26, 26, 26$  and 78 respectively (Darlington & Wylie, 1955). Favarger (1962) recorded  $n=15$  in *M. funkii* and  $n=x=23, 35$ , as well as  $2n=46, 70$  and 138 in *M. hybrida* subsp. *hybrida*. Favarger *et al.* (1979) recorded  $n=9$  in *M. geniculate*, and  $n=23, 35$

in *M. hybrida* subsp. *hybrida*. while Luque & Lifante (1991) reported  $2n=18$  in *M. hybrida* subsp. *hybrida*. Kamari *et al.* (1993) studied eight species of *Minuartia* and recorded  $n=15$  in *M. intermedia* and  $n=15$  in *M. recurva*. while Gucel (2013) recorded  $2n=30$  in *M. nifensis*. Dillenberger & Kadereit (2013) reported  $2n=30$  in *M. hamata* and  $2n= 22, 46$  and  $48$  in *M. hybrida*. Dillenberger & Kadereit (2013) reported values for *M. meyeri* ( $2n=30$ ), *M. montana* ( $2n=56$ ), *M. recurva* ( $2n=30$ ) and *M. picta* ( $2n=22$ ). Ghaffari & Kelich (2006) studied *M. lineate* and reported a value of  $n=10$ . The diploid chromosome number of *M. mesogitana* subsp. *mesogitana* was reported as  $2n = 22, 24$  (Çelebioglu & Favarger, 1984, 1990; Kamari *et al.*, 1993).

This study aims to investigate the chromosome number, karyotype, ideogram, and other detailed measurements of *Minuartia* taxa found in Iraq.

## Materials & Methods

Cytological studies were performed for nine taxa of *Minuartia*: *M. hamata*, *M. hybrida* subsp. *hybrida*, *M. intermedia*, *M. meyeri*, *M. picta* and *M. hybrida* subsp. *turcica*.

### Plant materials collection

Chromosome count and karyotype studies seeds of *Minuartia* were collected at multiple sites in northern Iraq (December 2013- May 2014). Flower buds were made after fixing with Carnoy's fixative using standard acetocarmine technique.

The seeds were germinated between moist Whatman's papers in Petri dishes at room temperature in the laboratory. The root tips were fixed in Carnoy's fixative absolute alcohol: glacial acetic acid (3:1) for 24 h in at 4°C overnight. Then, the root tips were stored in 70% ethyl alcohol in a refrigerator until examination. The root tips were hydrolyzed in

HCl (10%) for 12 min at 60°C. Root tips were stained with acetocarmine (2%) (Darlington & Lacour, 1969). Preparations were made using the squash method. Counting, measuring of chromosomes lengths, and karyotype analysis were done using slides contain the chromosomes at the metaphase stage of the mitosis. At least ten metaphase cells were used to determine chromosome numbers, photographed using a digital camera (DC-2) made in Taiwan.

### Karyotype description

The karyotype formula was determined by chromosome morphology based on centromere position according to Stace (1985), as given in table (1). The length of chromosomes was determined according to Avery *et al.* (1959), which classified chromosome length into five categories: very big ( $> 4 \mu\text{m}$ ), large (3.7-3.2)  $\mu\text{m}$ , medium (3.1-2.6)  $\mu\text{m}$ , small (2.1-1.5)  $\mu\text{m}$  and very small ( $< 1.5 \mu\text{m}$ ).

## Results

### Chromosome number

#### 1-*Minuartia hamata*

Mitotic metaphase chromosomes, karyogram, and monoploid ideogram of *M. hamata* are given in (Plate 1, figs. 1-5 and Plate 3, figs.1-2). The measurement data of these chromosomes are given in (Table 2). Metaphase analyses showed that the chromosome number of the species is  $n = x = 15$  (bivalent). Abnormal chromosomes were observed in early anaphase 1, such as bridge chromosomes. We also detected late chromosome division (Plate 1, fig. 5). Metaphase analyses showed that the chromosome number of the species is  $2n = 2x = 30 = 9m + 2sm + 3sacro + 1 \text{acro}$ . We detected five small or very small chromosomes in the remaining species.

Table (1): Karyotype formula.

Term	Centromeric	Arm ratio	Chromosome designation
M	Median region	1 - 1.7	Metacentric
Sm	Submedian region	1.7 - 3	Submetacentric
Subacr.	Subterminal region	3 - 7	Subacrocentric
Acro.	Terminal region	7 - $\infty$	Acrocentric
T	Terminal point	$\infty$	telocentric

The length of chromosomes varied from 3.40 to 8.58  $\mu\text{m}$ ; the average length of the chromosomes was 1.19  $\mu\text{m}$ .

### 2-*Minuartia hybrida* subsp. *hybrida*

Mitotic metaphase chromosomes, a karyogram and a monoploid ideogram of *M. hybrida* subsp. *hybrida* are given in (Plate 1, figs. 6-9 and 3, 3-4). The measurement data of

these chromosomes are given in (Table 3). Metaphase analyses showed that the chromosome number of the species is  $n=x=13$  as a bivalent chromosome. Our results show ring chromosomes in a different stage of meiosis division (Plate 1, figs. 6-7).

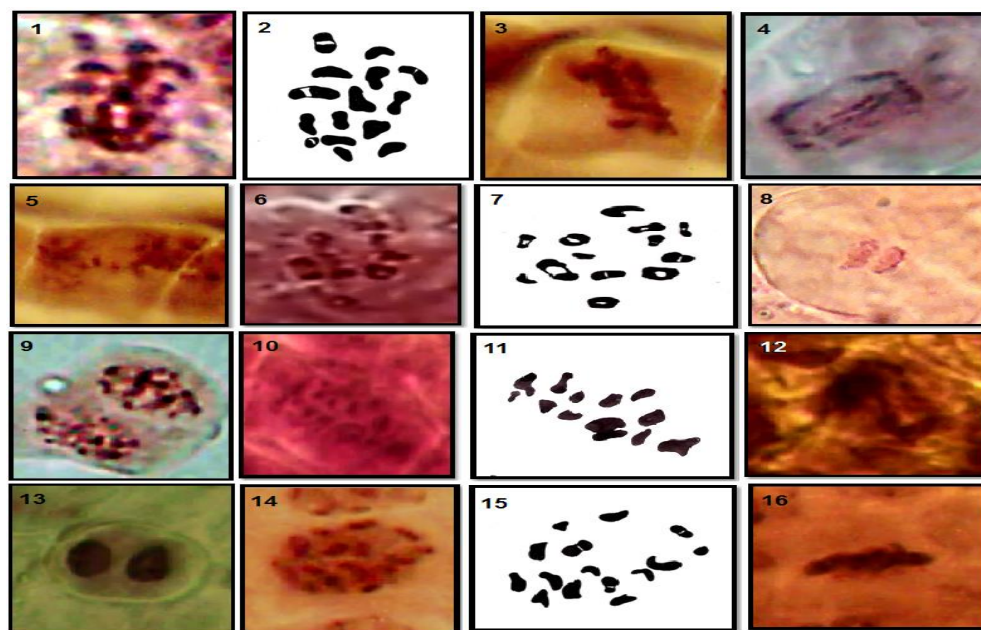
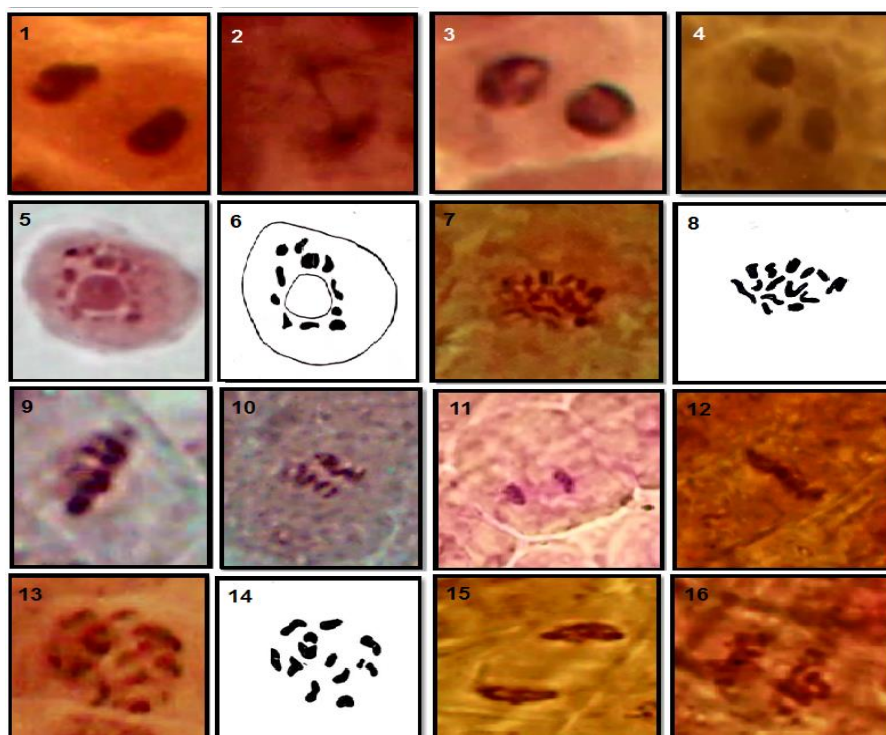


Plate (1): Microphotographs of meiotic division in *Minuartia* species (3200X). 1, 2- *M. hamata* ( $n=15$ ), 3- *M. hamata* (metaphase stage), 4- Bridge in *M. hamata*, 5- *M. hamata* (late chromosome in anaphase stage), 6, 7- *M. hybrida* subsp. *hybrida* ( $n=13$ ) and ring chromosome, 8- *M. hybrida* subsp. *hybrida* (early anaphase stage), 9- *M. hybrida* subsp. *hybrida* (metaphase stage), 10, 11- *M. intermedia* ( $n=13$ ), 12- *M. intermedia* (bridge), 13- *M. intermedia* (two nucleus in cell), 14, 15- *M. meyeri* ( $n=15$ ), 16- *M. meyeri* (metaphase stage).



**Plate (2):** Microphotographs of meiotic division in *Minuartia* species (3200X). 1- *M.meyeri* (Anaphase stage), 2- *M.meyeri* (bridge) 3- *M.meyeri* Two nuclei in one cell, 4- *M.meyeri* three nuclei, 5,6- *M.picta* (n=9), 7, 8- *M.picta* (n=14), 9- *M.picta* (metaphase stage), 10- *M.picta* (early anaphase stage), 11- *M.picta* (Anaphase stage), 12- *M. hybrida* subsp. *turcica* (Metaphase stage), 13,14- *M. hybrida* subsp. *turcica* (n=13), 15- *M. hybrida* subsp. *turcica* (Anaphase stage), 16- *M. hybrida* subsp. *turcica* (metaphase stage).

The formula is  $2n=2x=32= 9m + 3sm$ . The length of chromosomes varied from 2.95 to 8.33  $\mu\text{m}$ , (mean length 1.49  $\mu\text{m}$ ) (Table, 3).

### 3-*Minuartia intermedia*

Mitotic metaphase chromosomes, karyograms, and monoploid ideograms of *M. intermedia* are given in (Plate 1, figs. 10-13 & Plate 3, figs. 5-6). The measurement data of these chromosomes are given in (Table 4). Analysis of the metaphase stage showed that the chromosome number of the species is  $n=x=13$ . Abnormal chromosomes were observed in multiple stages of division, including bridge chromosomes and two nuclei in a single cell (Figs. 12 & 13). Metaphase analyses showed that the chromosome number of the species is  $2n = 2x = 26 = 9m + 3sm + 1sacro$ . Four chromosomes were small

or very small in the remain species. The length of the chromosomes varied from 4.01 to 10.40  $\mu\text{m}$ , (mean length 1.16  $\mu\text{m}$ ) (Table 4).

### 4-*Minuartia meyeri*

Mitotic metaphase chromosomes, karyogram, and monoploid ideogram of *M. meyeri* are given in (Plate. 1, figs. 14-16; Plate 2, figs. 1-5 & Plate 3, figs. 7-8). The measurement data of these chromosomes are given in (Table 5). Analysis of the metaphase stage showed that the chromosome number of the species was  $n = x = 15$  (bivalent). Bridge chromosomes, as well as two or three nuclei, were observed in cell division. Analysis of metaphases showed that the chromosome number of the species was  $2n = 2x = 30 = 12m + 3sm$ . All chromosomes were small sized. The length of

the chromosomes varied from 3.95 to 8.38  $\mu\text{m}$  (mean length of chromosomes was 1.11 $\mu\text{m}$ ) (Table 5).

#### 5- *Minuartia picta*

Mitotic metaphase chromosomes, karyogram and monoploid ideogram of *M. picta* are given in (Plate. 2, figs. 1-4). Analysis of the metaphase stage showed that the chromosome

number of the species is  $n = x = 11$  and 14 (bivalent).

#### 6- *Minuartia hybrida* subsp. *turcica*

Mitotic metaphase chromosomes, karyogram, and monoploid ideogram of *M. hybrida* subsp. *turcica* are given in (Plate 2, figs. 12-16). Analysis of the metaphase stage showed that the chromosome number of the species is  $n = x = 13$  (bivalent).

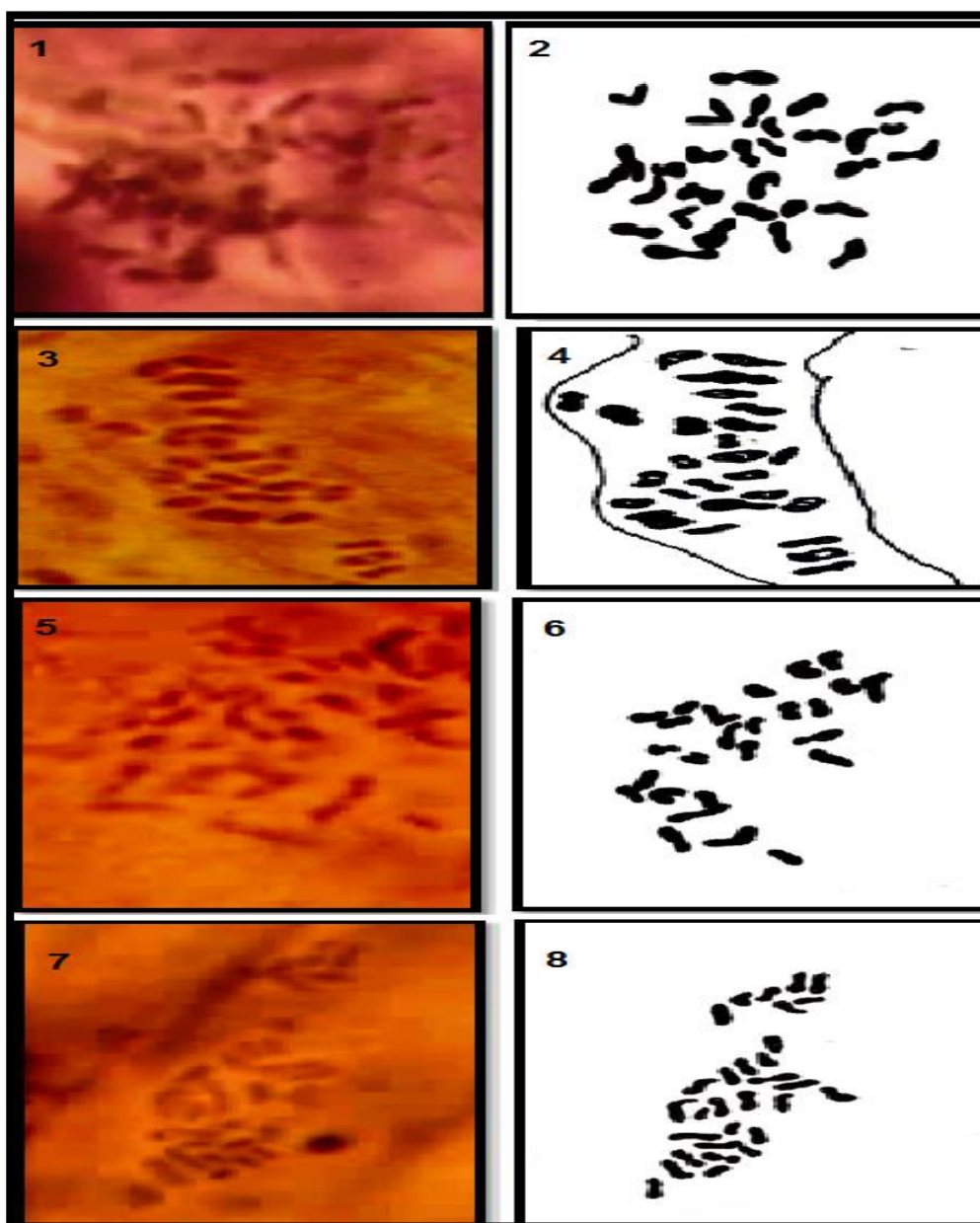


Plate (3): Microphotographs of meiotic division in *Minuartia* species (3200X). 1, 2- *M. hamata* ( $2n=30$ ), 3,4- *M.hybrida* subsp.*hybrida* ( $2n=26$ ), 5, 6- *M.intermedia* ( $2n=26$ ), 7, 8- *M.meyeri* ( $2n=30$ ).

**Table (2): The measurement data of the chromosomes of *M. hamata* ( $\mu\text{m}$ ).**

Chromosome pair	Average of chromosome length( $\mu\text{m}$ )	Long arm ( $\mu\text{m}$ )	Short arm ( $\mu\text{m}$ )	Arm ratio	Chromosome type	Length chromosome%
1	1.54	1.386	0.154	9	Acro	8.58
2	1.54	1.309	0.231	5.66	Sacr	8.58
3	1.54	1.15	0.38	3.02	Sacr	8.58
4	1.54	0.924	0.616	1.5	M	8.58
5	1.54	0.77	0.77	1	M	8.58
6	1.38	0.77	0.61	1.26	M	7.69
7	1.34	0.77	0.57	1.35	M	7.47
8	1.32	0.616	0.55	1.12	M	7.69
9	1.15	0.77	0.38	2.02	Sm	6.41
10	1.14	0.57	0.57	1	M	6.35
11	0.924	0.77	0.154	5	Sacr	5.15
12	0.77	0.539	0.231	2.33	Sm	4.29
13	0.77	0.385	0.385	1	M	4.29
14	0.77	0.385	0.385	1	M	4.29
15	0.61	0.38	0.23	1.65	M	3.40
	17.934					

**Table (3): The measurement data of the chromosomes of *M.hybrida* subsp. *hybrid*.**

Chromosome pair	Average of chromosome length ( $\mu\text{m}$ )	Long arm ( $\mu\text{m}$ )	Short arm ( $\mu\text{m}$ )	Arm ratio	Chromosome type	Length chromosome %
1	1.617	1.155	0.462	2.5	Sm	8.33
2	1.617	0.847	0.77	1.1	M	8.33
3	1.54	1.14	0.4	2.85	Sm	7.93
4	1.54	0.924	0.616	1.5	M	7.93
5	1.54	0.847	0.693	1.22	M	7.93
6	1.54	0.77	0.77	1	M	7.93
7	1.54	0.847	0.693	1.22	M	7.93
8	1.54	0.924	0.616	1.5	M	7.93
9	1.463	0.7315	0.7315	1	M	7.69
10	1.463	0.77	0.693	1.11	M	7.69
11	1.463	0.924	0.539	2.57	Sm	7.69
12	1.386	0.77	0.616	1.25	M	7.14
13	1.155	0.5775	0.5775	1	M	2.95
	19.404					

## Discussion

The cytogenetic characters especially chromosome size, number, and asymmetry are useful in plant cytotaxonomy. Caryophyllaceae comprises subfamilies Alsinoideae with  $x=6, 9, 10, 11, 12, 13, 14$  and 19 and Silenoideae with  $x=12-18$  (Jeelani *et al.*, 2011). Previous counts indicated the wide range of chromosome numbers ( $2n=11,$

$12, 13, 14, 15, 22, 23, 24, 26, 28, 30, 35, 36, 46, 48, 56$ ) in the genus *Minuartia* (Favarger, 1962; Fedorov 1974; Moore, 1977; Goldblatt & Johnson, 2003). Our cytological studies are based on six taxa comprising *Minuartia*. *M. picta* are showing the dibasic nature of the genus with  $x=11$  and 14, this result agreed with the findings of Dillenberger & Kadereit (2013) who recorded a value of  $x=11$ , and our

**Table (4): The measurement data of the chromosomes of *M. intermedia* ( $\mu\text{m}$ ).**

Chromosome pair	Average of chromosome length	Long arm	Short arm	Arm ratio	Chromosome type	Length chromosome %
1	1.58	0.79	0.79	1	M	10.40
2	1.57	1.15	0.42	2	Sm	10.33
3	1.52	0.92	0.65	1.41	M	10
4	1.51	0.77	0.75	1.02	M	9.94
5	1.4	0.79	0.61	1.29	M	9.21
6	1.39	0.77	0.62	1.24	M	9.15
7	1.17	0.77	0.4	1.92	Sm	7.70
8	1.15	0.58	0.5	1.01	M	7.57
9	1	0.79	0.21	3.76	Sacro	6.58
10	0.77	0.539	0.231	2.33	Sm	5.06
11	0.77	0.385	0.385	1	M	5.06
12	0.75	0.38	0.37	1.02	M	4.93
13	0.61	0.38	0.23	1.65	M	4.01
	15.19					

**Table (5): The measurement data of the chromosomes of *M. meyeri* ( $\mu\text{m}$ ).**

Chromosome pair	Average of chromosome length	Long arm	Short arm	Arm ratio	Chromosome type	Length chromosome %
1	1.40	0.7	0.7	1	M	8.38
2	1.40	0.78	0.62	1.25	M	8.38
3	1.38	0.69	0.69	1	M	8.26
4	1.32	0.66	0.66	1	M	7.90
5	1.30	0.65	0.65	1	M	7.78
6	1.25	0.79	0.46	1.71	Sm	7.48
7	1.25	0.77	0.48	1.60	M	7.48
8	1.15	0.8	0.35	2.28	Sm	6.89
9	1.15	0.57	0.57	1	M	6.89
10	1.15	0.8	0.35	2.28	Sm	6.89
11	1	0.5	0.5	1	M	5.99
12	0.77	0.539	0.231	1.67	M	4.61
13	0.76	0.385	0.385	1	M	4.55
14	0.75	0.38	0.37	1.02	M	4.49
15	0.66	0.33	0.33	1	M	3.95
	16.69					

work reported  $x=14$ , which considered a new chromosome number observed in *M. picta*.

In this study, the chromosome numbers, karyotypes, ideograms, and karyotype asymmetry degrees of *Minuartia* were determined. Also, chromosomal measurements of the *Minuartia* were reported. The chromosome number of *M.*

*hamata* is  $2n=30$ , which agrees with the literature (Celebioglu & Favarger, 1986; Dillenberger & Kadereit, 2013). Both species have small chromosomes (range, 8.58-3.40  $\mu\text{m}$ ). While *M. hybrida* subsp. *hybrida* reported  $2n= 26$  (Table 2); this same number of chromosomes was recorded in *M. meyeri* by Dillenberger & Kadereit (2013). Among

genetic variations, chromosome number is extremely variable (Eroglu & Per, 2016).

Karyotype asymmetry; can arise for multiple reasons; centromere position and the sizes of large and small chromosomes differ in karyotype asymmetry (Peruzzi & Eroglu, 2013). Karyotype asymmetry is an important parameter in karyological studies (Eroglu, 2015).

Here we detected meiotically abnormalities in the pollen grains different of multiple plant species, as has been reported in *Silene* (Sheidai *et al.*, 2008) and *Arenaria gypsophiloides* (Fadaei, 2010). All these meiotic abnormalities might lead to anomalous microsporogenesis and, in turn, to variable-size pollen grains and reduced fertility. Meiotically abnormal is the result of genetic factors (Ghaffari, 2006; Fadaei, 2010) and environmental factors (Nirmala & Rao,

1996), as well as genomic-environmental interaction (Baptista a-Giacomelli *et al.*, 2000).

The formation of cytomixis, chromosomal stickiness, unoriented bivalents, laggards, and bridges is considered the evolutionary significant in that it can lead to the production of plants with higher ploidy through polyploidization (Villeux, 1985). The cases of meiotic abnormalities found in some species of Caryophyllaceae family, such as *Lychnis senno* (Godo *et al.*, 2004), *Acanthophyllum laxiusculu* (Ghaffari, 2004), as well as *Silene*, *Stellaria* and *Arenaria* (Jeelani *et al.*, 2011).

Based on the results of this study and previous studies, we suggest draw a diagram cleared the emergence of the chromosome crew of the studied species (Fig. 1).

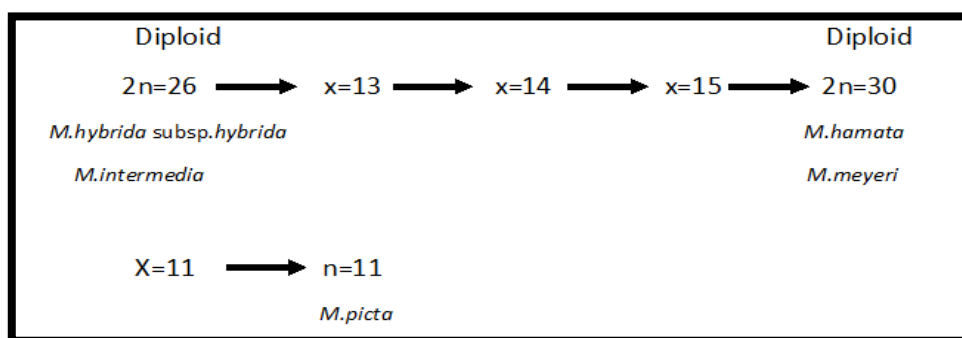


Fig. (1): Evolutionary pathway of several chromosomes in *Minuartia* species.

The *Minuartia* was represented by two basic numbers ( $x = 11$  &  $13$ ), which lead to an increase in chromosome number to  $x = 14$  and, subsequently,  $x = 15$ . *M. hybrida subsp. hybrida* was identified as diploid ( $2n = 26$ ). We detected values of  $2n = 30$  in *M. hamata* and *M. meyeri*.

## Conclusions

Based on the results obtained from the *Minuartia* taxa from populations located in the northern area of Iraq, we propose that the

two *Minuartia* species (*M. hamata* and *M. meyeri*) are haploid, with  $n = x = 15$  chromosomes, one species (*M. picta*) has haploid  $n = x = 11$  chromosomes, and the other species have 13 chromosomes. Karyotype analysis of *Minuartia* indicated that there are different levels of ploidy in this genus. The chromosomal number ( $x = 14$ ) reported here for *M. picta* is the first report of its kind. Polyploidy has a great effect on the phenotype



of the organism. A great morphological variation is also observed in these taxa. The morphological and genomic difference between these taxa can be indicated that the species can be formed more species by the hyperonization process. We suggest need for further phylogenetic and molecular studies to confirm the pungent of these taxa.

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