Research Article

Cytogenetic study and pollen viability of three populations of *Diplotaxis harra* (Brassicaceae) in Iran

Massoud Ranjbar[1,*] and Somayeh Karami[1]

1. *Department of Biology, Herbarium division, Bu-Ali Sina University, P. O. Box 65175/4161, Hamedan, Iran*

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Abstract

In this study, we examined the chromosome number, microsporogenesis, pollen fertility and distribution of cytotypes of *Diplotaxisharra* (Forssk.) Boiss. This is the first cytogenetic report of the taxon. Among three studied populations, two of them which belong to central regions of Iran showed aneuploid (x = 14) meiotic chromosome count while the other population existed at the diploid level (x = 13). It seems that the species with $x = 14$ are derived through aneuploid increase. The most prominent among these meiotic abnormalities was the chromatin stickiness which involved fragmented chromosome at different stages of meiosis. Consequently, these populations exhibited varying percentages of pollen sterility and pollen grains of smaller sizes. The aim of the present research was to study the male meiosis in detail, find the impact of chromatin stickiness in inducing meiotic aberrations and their consequent effects on pollen fertility and find out the distribution patterns of different cytotypes in Iran.

Keywords: Brassicaceae, Chromosome number, Cytotypes, *Diplotaxis*, Iran, Meiotic

Introduction

Diplotaxisharra is a genus of 2 species that belongs to the Brassicaceae in Iran, an economically important family with 321 genera and 3660 species (Al-Shehbaz, 2012; Hedge, 1968, 1980). The genus *Diplotaxis* belongs to tribe Brassiceae. The tribe consists of 46 genera and about 230 spp. characterized primarily by having conduplicate cotyledons, and segmented (heteroarthrocarpic) fruits, see Go´ mez-Campo, 1999, Appel and Al-Shehbaz, 2003, Warwick and Sauder, 2005). Most of cytological studies in the genus *Diplotaxis harra* have concerted on the chromosome counts, with a little work focused on detailed karyological criteria for taxonomic purposes. Results from cytological studies showed that there are three basic numbers and three ploidy level ($2n = 2x = 22$, $2n = 2x = 26$ and $2n = 2x = 38$) in the genus *D. harra* (Warwick and Al-Shehbaz 2006; Al-Shehbaz 1978; Al-Shehbaz and Al-Omar 1982; Malallah and Attia 2003; Snogerup 1985).
The present study reports meiotic chromosome number and behavior of three populations belonging to genus *Diplotaxis*and aims to increase the knowledge about patterns of chromosome numbers and meiotic behavior in the three populations of *D. harra.*

Materials and methods

Cytogenetic

Material for male meiotic studies were analysed in three populations of *Diplotaxisharra*which were collected from the wild plants growing in different localities of Markazi and Fars provinces, provinces in Iran in the months of April-May the year 2012 (Table 1). The voucher specimens are deposited in the Herbarium, Department of Biology, Bu-Ali Sina University, Hamedan. The young developing floral buds from healthy plants were fixed in modified Carnoy's solution in ethyl alcohol (96%), chloroform and propionic acid (6:3:2) for 24 h at room temperature and then stored in 70% ethyl alcohol at 4°C until used.

Chromosome counts and male meiotic analysis

Developing anthers from floral buds were squashed in 1% acetocarmine and preparations were studied for chromosome counts, and detailed meiotic behavior of pollen mother cells (PMCs) at early prophase-I, metaphase-I/II (MI/II), anaphases-I/II

*Corresponding authors E-mail:
massoud.ranjbar80@gmail.com

Table 1. Localities of the species used in this study.

Taxa	Voucher specimens	Altitude (m)	Location	Date	Collector name	Abbreviation
D. harra	30903	1400	Markazi: 35 km from Nubaran to Tafresh	26.5.2012	Ranjbar & Karami	HAR03
D. harra	30849	1434	Markazi: 30 km from Nubaran to Tafresh	26.5.2012	Ranjbar & Karami	HAR49
D. harra	29984	1450	Fars: Fasa, Azad university	27.4.2012	Ranjbar & Karami	HAR84

(AI/II), telophases-I/II (TI/II) and sporad stage (Wilson 1945). In populations with the normal meiotic course, a total of 95–127 PMCs were examined in determining the chromosome counts while in cytologically abnormal populations 20–50 slides prepared from different anthers/flowers (with 350–650 PMCs) were analysed in each case.

Pollenfertility

Pollen fertility was estimated through stainability tests for which anthers of mature flowers were squashed in glyceroacetocarmine mixture (1:1) dye. 800–1000 pollen grains were analysed in each case for pollen fertility and pollen size. Well-filled pollen grains with uniformly darkly stained cytoplasm were scored as fertile/viable while shrivelled pollen with unstained or poorly stained cytoplasm were counted as sterile/unviable. Pollen fertility was expressed as an average percentage of the stained pollen grains/total pollen grains analysed. Size of stained pollen grains was measured with occulomicrometer.

Photomicrographs

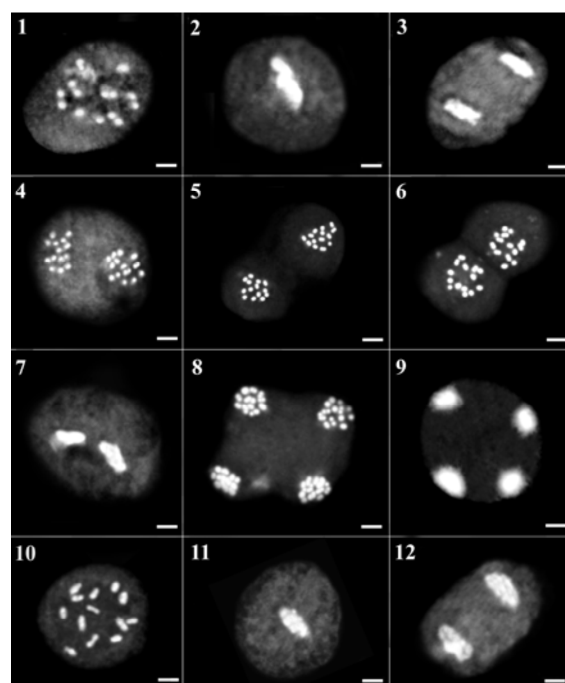
Chromosome spreads were analysed with Olympus light microscope and the best plates of chromosome counts, meiotic abnormalities, sporads and pollen grains (fertile, sterile) were photographed from the temporary mounts with an Olympus BX-51 microscope. Photographs of chromosomes were taken on an Olympus BX-51 photomicroscope at initial magnification of ×1000. Voucher specimens are kept at BASU, Hamedan, Iran (Table 1).

Results

Species Description

Diplotaxisharra (Forssk.) Boiss., Fig. 28.

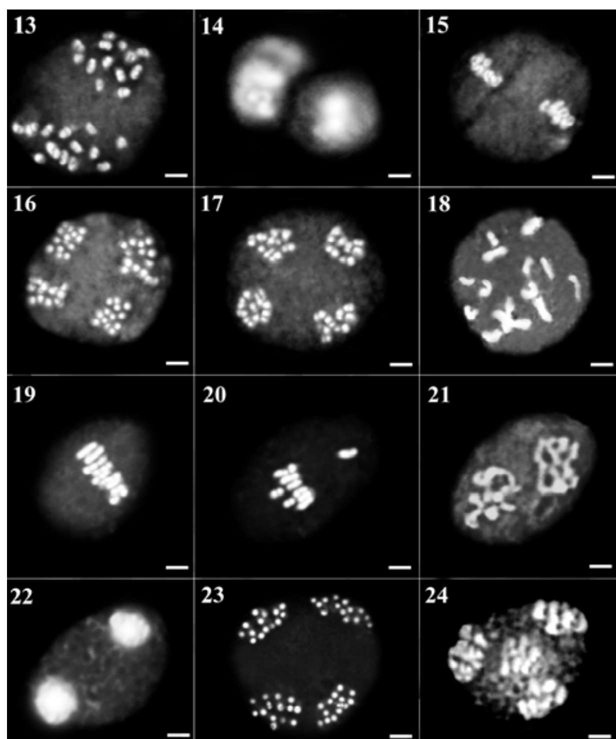
Annual to perennial herbs. Stems erect, 10-60 cm, simple or many-stemmed, glabrous or scattered simple hairs at lower part. Basal leaves petiolate, ca . 2.8 × 0.3 cm, obovate or broadly ovate, more or less dentate, apex acute, sparsely covered hairs, ca.



Figures 1-12. **R**epresentative meiotic cells in HAR49 and HAR84. (1) diakinesis ($2n = 2x = 28$) HAR49, (2) chromosome stickiness in metaphase I HAR49, (3) chromosome stickiness in anaphase I HAR49, (4-6) telophase I HAR49, (7) chromosome stickiness in metaphase II HAR49, (8) anaphase II HAR49, (9) telophase II HAR49, (10) diakinesis ($2n = 2x = 26$) HAR84, (11) chromosome stickiness in metaphase I HAR84, (12) chromosome stickiness in anaphase I HAR84. Scale bar = 1 µm.

1.2 mm long. Cauline leaves oblanceolate to oblong, 2.3–8.8 × 0.6–3.2 cm, apex acute, subsessile, entire. Inflorescence a raceme, up to 25 cm long, many flowered. Pedicels 10–15 mm long, erect to spreading, usually pendent when mature, becoming elongate in fruit and up to 24 cm long. Sepals spreading, inner not saccate, erect 4.8–6 × 0.8–2.2 mm, oblanceolate to oblong, acute, scarious margin, 0.2–0.4 mm long. Petals yellow or cream, distinctly longer than sepals, erect, 7–12.5 mm long, 2.5–5 mm broad, obovate, rounded at apex, claw differentiated from blade, shorter than sepals. Stamens 6, without appendages, slightly included,

erect; median filament pairs 4–6 mm long; anthers erect, linear, 2–3.2 mm long; lateral filament pair 3–5 mm long, anthers 2–2.7 mm long. Fruits 30–50 × 2.5–3 mm long, linear, compressed siliqua, 1-veined, beak very short or ca. 1 mm, terete, erect, gynophore ca. 2–3 mm long, glabrous. Stigma prominent, 2-lobed; style absent. Ovary with very many (up to 100) ovules. Seeds numerous, arranged in two rows in each cell. Cotyledons longitudinally folded.



Figures 13-24: Representative meiotic cells in HAR84 and HAR03. (13-14) telophase I HAR84, (15) metaphase II HAR84, (16) anaphase II HAR84, (17) teloaphase II HAR84, (18) diakinesis ($2n = 2x = 28$) HAR03, (19) metaphase I HAR03, (20) metaphase I with fragmented chromosome HAR03, (21) anaphase I HAR03, (22) teloaphase I HAR03, (23) anaphase II HAR03, (24) teloaphase II HAR03. Scale bar = 1 µm.

Cytogenetics

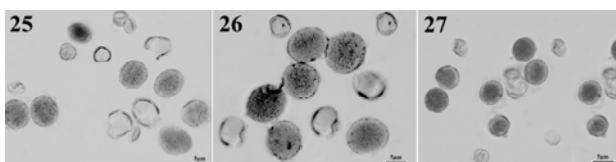
Chromosome numbers and meiotic behavior were determined in three individuals belonging to three populations of one species. They were similar in life history, breeding system, ecology, and geographical distribution in Iran. A total of 542 diakinesis/metaphase I (D/MI); 318 anaphase I/telophase I (AI/TI); 27 metaphase II (MII) and 505 anaphase II/telophase II (AII/MII) cells were analyzed. Only one population growing in the Fars (1450 m) existed at diploid level (based on $x = 13$) as confirmed from the presence of 13 medium sized bivalents in the PMCs at MI (Fig. 10). These bivalents showed regular segregation during anaphase I. Further meiotic course was also regular resulting into normal tetrad formation, nearly percent pollen fertility and uniform sized pollen grains. The aneuploid cytotype has been found to be more common as confirmed from the presence of meiotic chromosome number of $x = 14$ in two out of the three populations scored presently from the different localities in the Iran. These aneuploid individuals in the populations unequivocally showed the presence of 14 bivalents in the PMCs (Figs. 1, 18). All taxa studied here displayed regular bivalent pairing and chromosome segregation at meiosis. However; some meiotic abnormalities were observed. The meiotic irregularities observed in different *D. harra* included the occurrence of varied degree of fragmented chromosome to poles and chromosome stickiness (Table 2).

Table2. Characterization of meiotic behavior and pollen viability in populations of *Diplotaxisharra*

Meiotic characters	HAR49	HAR84	HAR03
Cell number	360	507	628
D/MI	94	143	305
% D/MI	26.11	28.20	51.75
% Fragmented chromosome	0	0	5.27
AI/TI	32	183	103
% AI/TI	8.88	36.09	16.40
% Chromosome stickiness	60	11.18	0
MII	2	25	0
% MII	0.55	4.93	0
AII/TII	184	139	182
% AII/TII	51.11	27.41	28.98
N	14	13	14
% Pollen viability	46.37	54.21	49.37

Pollenviability

The results of the comparison between the meiotic behavior and pollen viability showed the highest (54.21) and lowest (46.37) percentages of the stained pollens in HAR84 and HAR49; respectively. This result indicates that irregularities observed at meiosis probably have a direct relation with species fertility. The pollen viability of examined species is described in Table 2 and illustrated in figures 25, 26 and 27. Chromosome stickiness coupled with associated meiotic abnormalities and consequent abnormal microsporogenesis resulted into high pollen sterility (Table 2) and smaller sized pollen grains (Fig. 25).

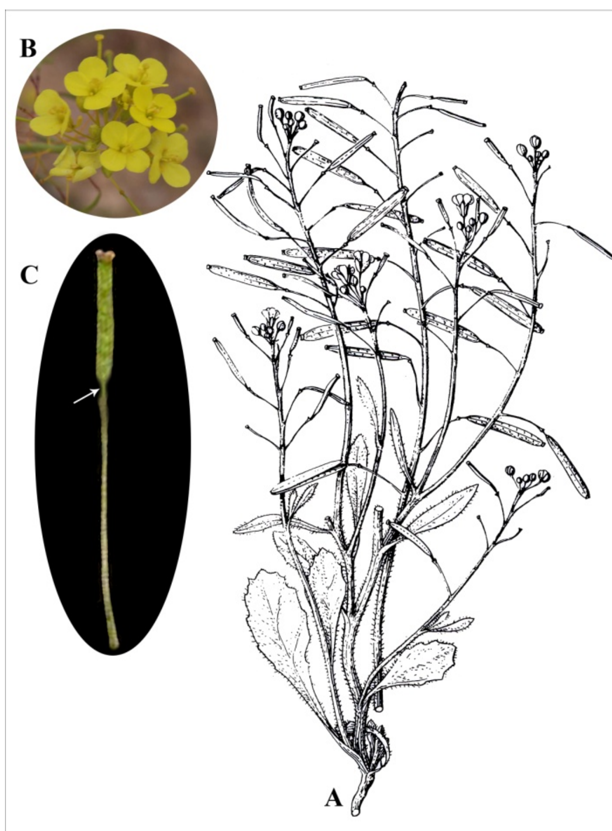


Figures 25-27: Pollen viability. (25) sterile pollen grains among fertile pollen grains in HAR49, (26) HAR84, (27) HAR03. Scale bar = 5 µm.

Discussion

Chromosomenumber and meiotic behavior

All populations are diploid and possess $2n = 2x = 26$, $2n = 2x = 28$ chromosome number, which is consistent with the proposed basic number of $x = 13$. The present diploid ($2n = 26$) and aneuploid ($2n = 28$) chromosome counts for the species from this region of the Iran, explored for the first time, agree with the earlier reports from other regions of Iran. Based on $x = 13$ another proposed basic number for



Figures 28. *Diplotaxisharra* (Forssk.) Boiss. (A) Plant, (B) Close up flowers, (C) Close up glabrous fruits and gynophore [illustration 28A through the courtesy of Flora of Iraq 4 (2): 860].

Diplotaxisharra a diploid (Warwick and Al-Shehbaz 2006). It is thus apparent that the species exhibits considerable degree of variability in chromosome number in the Iran. In addition to the presence of intraspecific cytotypes, the species also showed the existence of diploid and aneuploid chromosomal races ($2n = 26$, $2n = 28$) at basic number of $x = 13$ and $x = 14$. Consequently, the individuals of diploid and aneuploidy cytotypes of *D. harra* can't be distinguished from each other in the field. Meiosis is highly coherent and the process is genetically programmed, which comprises of pairing homologous chromosomes, crossing over, reduced in chromosome number, and lacking of S period between the two divisions. Similar to any other biological process, all the sequential steps involved in meiosis are controlled by a large array of genes (Ramana, 1974; Mok and Peloquin, 1975; Mok et al., 1976; Koduru and Rao, 1981; Falistocco et al., 1995). Mutation in any of these genes that govern micro or megasporogenesis from pre-meiotic to post meiotic events can lead to serious anomalies in the whole process resulting in the genetically aberrant end products having an adverse impact on fertility and overall reproductive efficiency of the species (Lattoo et al., 2006).

Stickychromosomes

Sticky chromosomes were observed in two studied populations with different frequencies (Figs. 2, 3, 7, 11, 12, Table 2). Chromosome stickiness may be caused by genetic and environmental factors, and several agents have been reported to cause chromosome stickiness (Pagliarini, 2000). Chromosome stickiness also resulted in the formation of pycnotic chromatin at earlier stages of prophase I and chromatin bridges at anaphase/telophase. The normal functioning of spindle apparatus is crucial for chromosome alignment during metaphase and correct segregation of chromosomes to poles (Shabrangi et al., 2010). Disturbed spindle apparatus orientation may have resulted in scattered and disoriented chromosomes in the meiocytes.

Genetic as well as environmental factors have been considered as the reason for chromosome stickiness in different plant species (Nirmala and Rao, 1996). They are characterized by their agglomeration in different stages of the cell cycle and can occur due to the presence of mutant genes or abiotic factors as X-rays (Steffensen, 1955; 1956), gamma irradiation (Rao and Rao, 1977; Al Achkar et al., 1989), temperature, herbicides (Badr and Ibrahim, 1987) and some chemical elements present in soil (Steffensen, 1956; Zanella, 1991; Caetano-Pereira et al., 1995). Another hypothesis is that chromosome stickiness may be caused by a failure or a bad functioning in one or two types of non-

histone chromosomal proteins (Peres Kiihl et al., 2011). The highest frequency of chromosome stickiness of anaphase I and telophase I cells was observed in population of HAR49 (Figs. 2, 3, 7, Table 2). The lowest degree of chromosome stickiness occurred in population of HAR84 (Figs. 11, 12, Table 2).

Fragmented chromosomes (Precocious migration)

There was another abnormality in metaphase I (Fig. 20). The highest frequency of fragmented chromosomes of metaphase I cells was observed in HAR03 population (Table 2). The highest and lowest abnormalities were seen in populations of HAR49 and HAR03, respectively.

Pollenviability

Furthermore, many abnormalities affecting plant fertility or causing total male sterility have been detected during the evaluation of meiotic behavior in some species. There is a difference in the pollen grain size of the diploid cytotype and in the two populations (Fig. 26) of the aneuploidy cytotype where the typical pollen grains were similar in smaller size as that of the diploid cytotype. So, the aneuploid increase can be concluded in the pollen grain size of populations in HAR49 and HAR03.
Different sized pollen grains in other populations of the aneuploidy cytotypes are the product of various meiotic abnormalities abnormal microsporogenesis. Consequently, very high pollen sterility and fertile pollen grains of two heterogeneous sizes were resulted. This result is predictable based on meiotic behavior data and of the lowest percentages of irregularities in these populations (Table 2). In contrast, a low percentage of pollen viability (46%) in population of HAR49 can be explained by having a high percent of sticky chromosomes. In this population a relatively high frequency of chromosome stickiness in different stages of meiosis and consequently, low pollen viability was observed. So, it can be concluded that sticky chromosomes affects the meiotic course considerably and results in reduced pollen viability, also showed that there is a direct relationship between occurrence of sticky chromosomes and reduced pollen viability. The pollen viability of examining populations is described in Table 2 and illustrated in figures 25, 26 and 27.

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