African Journal of Agricultural Research Vol. 7(33), pp. 4589-4595, 28 August, 2012
Available online at http://www.academicjournals.org/AJAR
DOI: 10.5897/AJAR12.052
ISSN 1991-637X ©2012 Academic Journals

Full Length Research Paper

Karyotype analysis and meiotic behaviors of *Ducrosia anethifolia*: The first case study

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Accepted 4 June, 2012

The medicinal plant *Ducrosia anethifolia* (DC) *Boiss* is an annual herb of the Apiaceae family. It originated from Iran and it is used to treat headache and backache in traditional medicine. Mitotic study and meiotic behavior of chromosomes are presented for the first time for *D. anethifolia* in this study. *D. anethifolia* possessed n = 11 (2n = 2x = 22) chromosome number forming mainly both rod and ring bivalents with some amount of quadrivalents and univalents. Karyotype analysis according to Stebbins categories, placed chromosomes of this plant in symmetric class of 2A, indicating a symmetric karyotype. The types of chromosomes were metacentric and submetacentric with one pair of satellite chromosomes. A variety of abnormal chromosomal behaviors including chromosomal stickiness, bridge, laggards, micronucleus, mass chromatin and early chromosome migrations were observed, albeit with low frequency. Tetrads with four equal-sized cells were considered normal and any deviation as abnormal. Stain-ability of pollens as an estimation of their fertility was ninety-seven percent. In this study we had few meiotic abnormalities and, as a consequence, a high pollen fertility. Well-filled pollen grains with stained nuclei were regarded as apparently fertile while shriveled, smaller microspores and unstained pollen were counted as sterile. The study reveals that meiosis in this species is normal, with high meiotic index and regular bivalent formation in most studied cells.

Key words: *Ducrosia anethifolia*, karyotype, meiosis, pollen fertility.

INTRODUCTION

Ducrosia anethifolia Boiss of the family Apiaceae has a limited distribution, which was included mainly in Afghanistan, Iran, Iraq and Pakistan. Natives in these geographic regions is exploiting this plant to feed sheep and camels and it has been used for medicinal and culinary purposes. In Iran, the plant is used to increase the smell of foods and drinks as an additive. In traditional medicine also, it is used to treat catarrh, headache and backache; its seeds are also given to children as an infusion in case of colic (Janssen et al., 1984). Basic information on meiotic behavior and pollen viability estimations are important, since the germplasm characterization is used to determine the genetic variability, study biodiversity, and evolution processes (Silva et al., 2004). Knowledge of karyotype is an important prerequisite for effective plant genetic and breeding studies. Since there is no well documented cytogenetic study on *Ducrosia* species throughout the world, the aim of the present paper was to investigate the karyotype of *D. anethifolia* and to furthermore reveal the basic valuable cytological information for breeding programs.

MATERIALS AND METHODS

The biological material used in this study was collected between April and May 2010 from the Institute of Medicinal Plant in Iran.

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Table 1. The morphology of chromosomes in *D. anethifolia*.

No.	TCL (micron)	RL %	LA (micron)	SA (micron)	AR	CI
1	7.3533	11.878	4.0547	3.2987	1.2292	0.4486
2	6.8818	11.116	4.4050	2.4768	1.7784	0.3599
3	6.5221	10.535	3.6740	2.8480	1.2900	0.4367
4	6.0327	9.7452	3.8894	2.1434	1.8145	0.3553
5	5.5805	9.0146	3.1933	2.3872	1.3376	0.4278
6	5.2933	8.5507	3.2722	2.0211	1.6190	0.3818
7	5.2088	8.4142	2.6643	2.5446	1.0470	0.4885
8	5.1691	8.3501	3.5510	1.6182	2.1944	0.3130
9	5.0887	8.2202	3.6748	1.4139	2.5990	0.2779
10	4.6417	7.4981	2.6810	1.9608	1.3672	0.4224
11	4.1326	6.6758	2.1529	1.9798	1.0874	0.4791

No, Chromosome pair number; TCL [LA+SA], total chromatin length; RL, relative length; LA, long arm; SA, short arm; AR [LA/SA], arm ratio; CI [SA/ (LA+SA)], centromeric index.

Cytological analysis

Cytological observations were made on mitotic cells of root tip meristems obtained from a 10 day-old-seedling. Roots with a length of approximate 0.5 to 1 cm were excised between 8:00 and 10:00 a. m and were treated in 8-hydroxyquinoline 0.02 M solution for 4 h at 17.5°C in darkness, and washed water for five minutes. Pre-treated root tips were fixed in 3: 1 ethanol: glacial acetic acid for 24 h at 4°C. Samples were hydrolyzed in 1 M HCl for 4 to 5 min at 60°C and washed. Then staining was carried out with aceto-orcein dye (2%) for 2 h at room temperature followed by squashing a little bit of rot tip on standard slides. The 10 best metaphasic plates were selected and prepared images analyzed by Micromeasure 3.3 software (Reeves et al., 2000); Then, the following parameters were estimated: Long arm (LA), short arm (SA), total chromatin length (TCL), relative length percentage (RL%), arm ratio (AR), Centromeric index (CI), (Bazzichelli, 1967; Martinoli and Ogliotti, 1970). Karyotype asymmetry was estimated by some different methods such as the total form percentage (TF %) (Huziwara, 1962), difference of relative length (DRL), intra-chromosomal asymmetry index (A_1), inter-chromosomal asymmetry index (A_2) (Romero Zarco, 1986) and the percent of symmetry index (SI %). Also, karyotypic evolution has been determined by the methods of Stebbins (1971). The nomenclature used for the description of chromosome morphology is that proposed by Levan et al. (1964).

Meiosis

For meiotic studies, young and immature flower buds were collected from 10 randomly selected plants and fixed in freshly prepared Carnoy's solution (3: 1 ethanol: acetic-acid) for 24 h. Flower buds were washed and preserved in 70% ethanol and stored at 4°C. Cytological preparations used here were staining by 1% aceto-carmine dye and squashing. The gametic chromosome number was determined in at least 10 cells. Observations on chromosome pairing at various stages of meiosis were recorded. Meiotic indices were estimated following Love (1951). To determine the meiotic index, we evaluated 100 tetrads. Tetrads with 4 equal-sized cells were considered normal and any deviations as abnormal. The meiosis tests included observations of chromosome pairing at diakinesis and metaphase I in PMCs. On the basis of chromosome pairing data, the mean number of bivalents, modal numbers of bivalents and univalents were calculated. The number of rod and ring shaped bivalents and the presence of univalents was estimated. Pollen stainability was used to indicate pollen viability. Anthers at proper stage were collected from the 10 randomly selected plants. Pollen stainability as a criterion of fertility was determined by staining at least 300 pollen grains with 2% aceto-orcein for about 30 min. Round-complete pollens which were stained were taken as fertile, while small and shriveled unstained pollen grains were scored as sterile (Sheidai et al., 2000).

RESULTS

Karyotype analysis

Chromosome numbers were obtained from somatic mitosis of root tips of the *D. anethifolia* and the karyotype characteristics were presented in Table 2. The diploid chromosome number of *D. anethifolia* was found to be 2n = 22 (Figure 1). A pair of small satellites was observed on the shorter arms of the ninth chromosome pair. The ideogram is shown in Figure 2. Most of its chromosomes were metacentric and submetacentric pairs and had a karyotypic formula of 7 m + 4 sm. The chromosomes size ranged from 4.1326 to 7.3533 µm and the ratio of the longest to shortest chromosome was 1.7793. The arm ratios ranged from 1.0470 to 2.599. The total haploid length of the chromosomes (TCL) was 61.905 µm. The karyotype had relatively high symmetry and was classified as 2A (Stebbins, 1971). Data is given in Table 1. Chromosomes were numbered from 1 to 11 in order of their decreasing total length.

Meiotic behavior

In this species, most stages of meiosis was regular and showed 11 bivalents at metaphase I by normal chromosome segregation (11 to 11) at Anaphase I. *D. anethifolia* formed both ring (68.18%) and rod (30.36%)

Table 2. Karyotype characteristics in *D. anethifolia*.

Karyotype formula	Percentage of chromosome with arm ratio >2	SC	TF%	DRL	SI	A1	A2
7 m + 4 sm	18.18	2A	39.88	5.202	56.20	0.68	0.60

2n- Somatic chromosome number, SC, symmetry classes of Stebbins, A_1[1-Σ(SA/LA)/n], intra-chromosome asymmetry index, A_2[S d/X], inter-chromosome asymmetry index, TF% [(ΣSA/ΣTL)*100], total form percentage, DRL[Max RL% - Min RL%], difference of relative length, SI, symmetry index percentage.

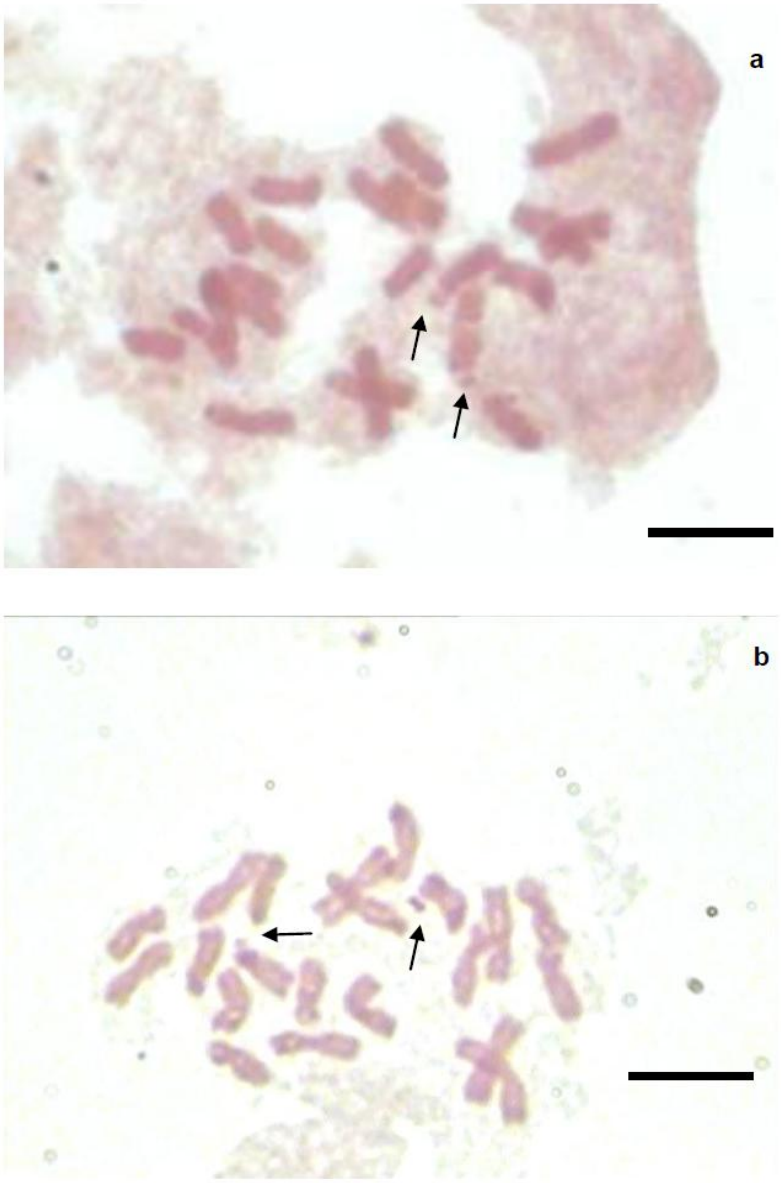

Figure 1a, **b.** Somatic chromosomes of *D. anethifolia* 2n = 22. Arrowheads show satellites, Bar corresponds to 10 µm.

bivalents. Univalents and quadrivalents were also observed at low frequency (1.45%) in diakinesis of Prophase I Figure 3; this is interesting since diploid species are expected to form only bivalents.

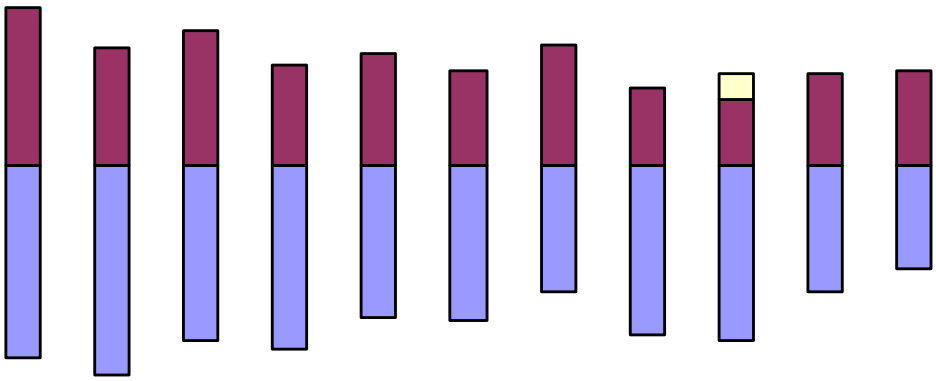

Figure 2. Idiogram of somatic chromosomes of *D. anethifolia*.

A variety of irregular meiotic behaviors were observed at different stages that include the presence of laggard chromosomes in various stages (8%), chromosome bridges in Anaphase I and II (3.6%), precocious chromosome migration (2.3%), micronuclei in tetrads or microspores (1.8%) and micronuclei in other stages (6%). Also, the test for pollen fertility showed a low percentage of sterile pollen grains and the percentage of normal tetrads and pollen viability was 93 and 97% respectively (Figure 3 and 4).

DISCUSSION

Our study confirmed previous ones in which the somatic chromosome number of *D. anethifolia* was noted as 2n = 22 and with basic chromosome number x = 11 (Cartier, 1983). To our knowledge, the karyotype analysis of *D. anethifolia* reported for the first time in the present study showed that the chromosomes were metacentric and submetacentric. Furthermore, the arm ratio of the most chromosome pairs ranged from 1.0470 to 1.8145 µ while only a few other chromosome pairs exceeded 2.0 µ. This showed that the intrachromosomal symmetry in *D. anethifolia* is high. According to Stebbins (1971), primitive plants have symmetric karyotypes and as such, karyotype analysis reveals that *D. anethifolia* is a primitive toxin. On the other hand, by using the Romero-Zarco asymmetry indices, we determined the asymmetric karyotype in the *D. anethifolia* which had similar Stebbins classes of symmetry. Our results confirmed the presence of X = 11 chromosome number like the most other species of Apiaceae family. This study reveals that *D. anethifolia* had a predominantly regular meiotic behavior and 11 regular bivalents were observed at diakinesis and metaphase I in most cells analyzed, although, some abnormalities were observed in meiotic stages. Some laggard univalents were seen in some cells (8%) and this occurrence might be due to the delayed terminalisation, stickiness of chromosomal ends or because of failure of the chromosomal movement (Baht et al., 2007). Low frequencies of bridge (3.6%) at Anaphase I was also observed. As the consequence of the laggard chromosomes and precocious univalent migration, micronuclei were observed in Telophase I, Anaphase II, which normally remain until the tetrad stage (1.8%) and may result in variation in number and size of pollen grains (Koduru and Rao, 1981).

Sheidai et al. (2007) studied some populations of *Foeniculum vulgare* (Umbelliferae) in Iran. All mentioned populations possessed n = 11 (2n = 2x = 22) chromosome number forming mostly both ring as well as, rod bivalents with some amount of univalents and quadrivalents. Forming quadrivalents in the diploid species that is expected to form only bivalent is known as the consequence of the occurrence of heterozygote translocations.

Morphological observations of the pollen grains in *D. anethifolia* exhibited the occurrence of smaller sized pollen grains. According to Xing-Feng et al. (2005), the smaller microspores that have not suffered the normal mitosis will be eventually unfertile. Another abnormal phenomenon in some cases, were the extra chromatin masses present in the PMCs that do not pair with the main chromatin and remain in the cell as a separate mass. According to Singhal and Kumar (2008), as a result of chromatin migration, PMCs showed extra chromatin masses lying away from the main chromosome complement. The fate of such additional masses of chromatin is not known, but they probably form micronuclei or micro-pollen as suggested by Bhat et al. (2006).

The effect of meiotic irregularity can be evaluated by recording pollen and plant sterility. Fertility depends on the efficiency of the meiotic process (Bione et al., 2000). Studies on different plant species have shown that the decline in seed production is correlated with meiotic irregularities (Moraes-Fernandes, 1982; Pagliarini, 1990; Pagliarini and Pereira, 1992; Consolaro et al., 1996; Khazanehdari and Jones, 1997). In most of the species,

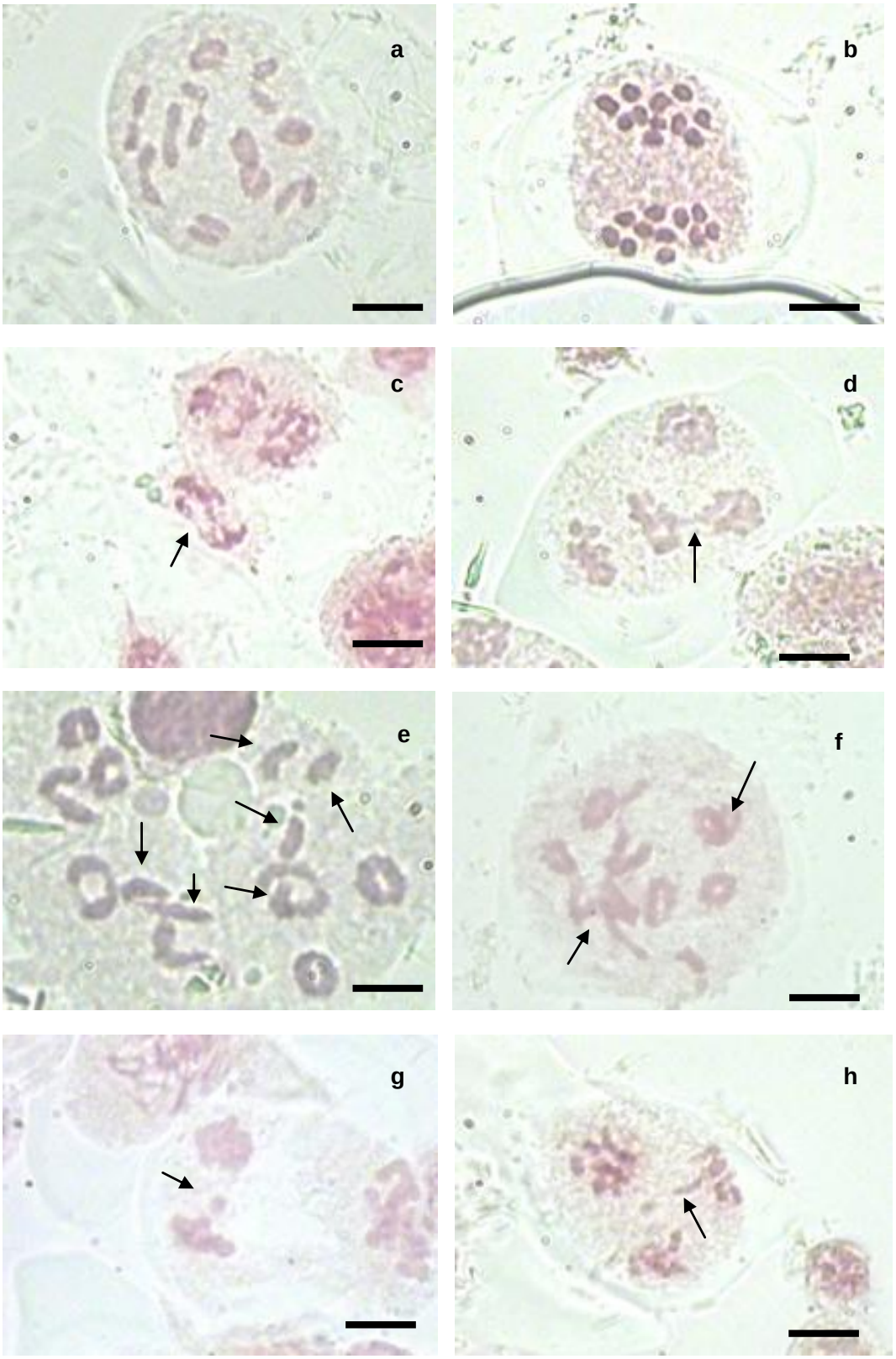

Figure 3. Meiotic cells of *D. anethifolia* a: diakinesis with11 normal rod and ring bivalents; b: Anaphase I, showing (11-11) segregation; c: Chromosomal bridge (arrow indicating) at Anaphase I; d: Chromosomal bridge (arrow indicating) at Anaphase II; e: Diakinese with six univalent (arrow indicating); f: Diakinese with two quadrivalents (arrow indicating); g: Laggard chromosome at Telophase I (arrow indicate); h: Triad formation (unreduced cell) with laggard chromosome (arrow indicate). Bar = 10 µm.

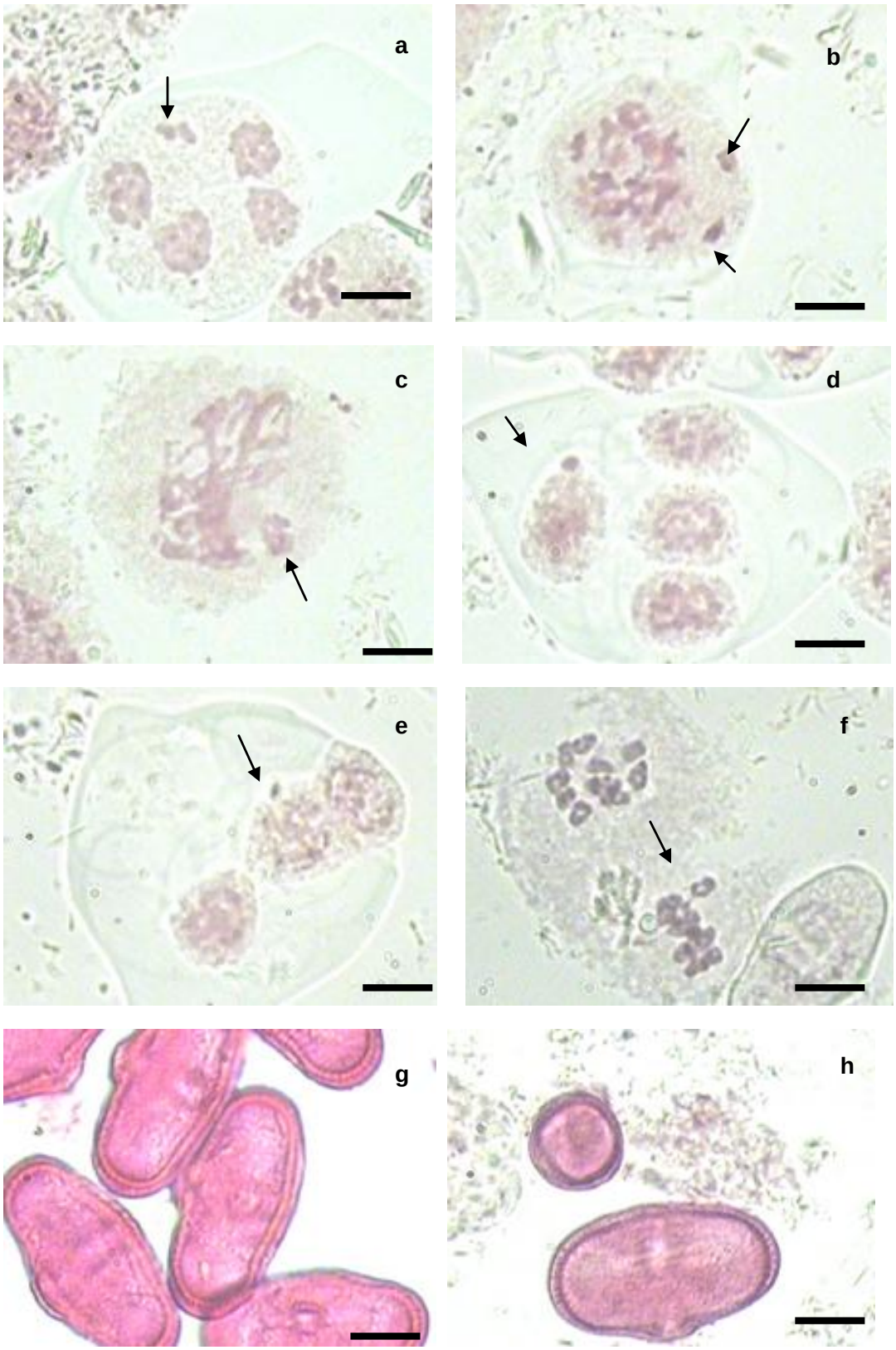

Figure 4. Meiotic cells of *D. anethifolia* a: Micronucleus at Anaphase II to Telophase II (arrow indicating); b: microspore showing two micronuclei (arrow indicating); c: PMCs showing extra chromatin masses (arrow indicating); d: Tetrad showing a micronucleus in one microspore (arrow indicating); e: Triad formation with one micronucleus in one microspore (arrow indicating); f: Precocious chromosome migration g: equal-sized pollen grains; h: different-sized pollen grains. Bar = 10 µm.

pollen fertility expressed a close relationship with meiotic abnormalities (Bione et al., 2000; Cavalcanti et al., 2000; Singhal and Kumar, 2008). Love (1951) recommended measuring meiotic instability using the meiotic index, and in *D. anethifolia*, the percentage of normal tetrad was expressed as the average meiotic index, which was 93% and pollen viability was 97%. Therefore, the high pollen viability reveals that irregularities observed at meiosis probably are not significant in terms of species fertility.

Conclusion

Cytological studies within Apiaceae have been limited, and often, a little more than the chromosome number is known for most umbellifer vegetables and the majority of the karyological data refers to the carrot subfamily (Iovene et al., 2008). Present results allowed us to compare the karyotype of *Ducrosia* species by other diploid species in Apiaceae family for the first time. It was believed that the basic chromosome number in Apiaceae is x = 11 and that a descending diploid series (containing x = 10 and 9) could be taken from it (Moore, 1971; Pimenov et al., 2003). According to Iovene et al. (2008) who compared karyotype analysis in some *Daucus* species with other Apiaceae, most of them had at least one subtelocentric pair of chromosomes, but the majority of their chromosomes was metacentric and submetacentric, with arm ratio < 2: 1 and as such were classified as 2A for karyotype symmetry.

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