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Short communication

Chromosome numbers and karyotype features of *Phlomis olivieri* Benth. (Lamiaceae) from Iran

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Abstract – Chromosome numbers were determined in ten accessions of *Phlomis olivieri* Benth. (Lamiaceae). The seeds were collected from natural habitats in the west of Iran. Chromosome numbers of all accessions were 2n=2x=20. The chromosomes of accessions were metacentric or submetacentric, ranging in length from 2.66 to 8.604 µm. According to the average values of ten accessions, the karyotype of this species consists of 10 pairs of metacentric chromosomes. An ideogram was depicted for the species. This is the first report on the chromosome number and karyotype analysis of *P. olivieri* from Iran.

Keywords: chromosome count, Iran, karyotype feature, Labiatae, *Phlomis*

Introduction

The genus *Phlomis* L. is one of the largest genera of subfamily Lamioideae (Lamiaceae) with more than 100 recognized species distributed in Asia, southern Europe and northern Africa, which have been divided into two main sections: *Phlomoides* (Moench) Briq. and *Phlomis* (Rechinger 1982, Albaladejo et al. 2005). The diagnostic character for separating the sections is corolla shape. Species of the section *Phlomis*, which have a corolla with a curved upper lip and trifid lower lip with large median and smaller lateral lobes, differs from species of section *Phlomoides* that have corolla with straight upper lip and trifid lower lip with sub equal lobes (Azizian and Moore 1982). In Iran, this genus is represented by 19 species (10 species are endemic) including *P. olivieri* Benth. (sect. *Phlomis*) which grows wild in north, west and central Iran (Rechinger 1982, Jamzad 2012). Recently, the taxonomic value of the indumentum as well as the anatomy and palynology of this species were investigated (Yousefi et al. 2014).

There is little cytological information on the genera, though there have been chromosome counts of some species of *Phlomis* by workers such as Wagner (1948), Reese (1953), Strid (1965), Zhukova (1967), Chuksanova and Kaplanbekova (1971), Azizian and Cutler (1982), Strid and Andersson (1985), Aparicio (1997), Aparicio and Albaladejo (2003), Ghaffari (2006) and Özdemir et al. (2014). The chromosome number of the species of *Phlomis* varies from 2n=10 to 2n=46 (Goldblatt and Johnson 1979).

Phlomis olivieri is a perennial herb species distributed in Iran and Iraq. This species grows on mountainous regions, adjacent to rocky slopes, steppe vegetation and the overgrazed rangeland soils of the Irano-Turanian region and the Hyrcanian district of Iran (Jamzad 2012) and could be considered one of important destroyed rangeland indicators together with *Stachys inflata* Benth. (Mozaffarian 2005). The aim of this paper is to determine the chromosome number and karyotype features of *P. olivieri* for the first time.

Materials and methods

Plant samples of *P. olivieri* Benth. were collected in wild populations in the west of Iran. Voucher specimens of all the materials studied were deposited in the herbarium of Isfahan University, Iran. The locality, collectors and dates of ten accessions are shown in Table 1. Root-tip meristems were obtained from wild collected seeds germinated on wet filter paper in Petri dishes at room temperature in the dark. They

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Tab. 1. Localities and chromosome numbers of the investigated accessions in *Phlomis olivieri*.

No.	Location and collection number	Geographical character	Altitude (m)	Chromosome number	Date of collection
1	Hamedan Province: Ganjnameh, before Mishan plain, Yousefi 19003.	34°46'03.89"N 48°25'43.67"E	2550	2n = 20	4 June 2011
2	Hamedan province: Asadabad, around Taj abad sofla village, Yousefi 19013.	34°52'49.62"N 48°12'44.58"E	1994	2n = 20	4 June 2011
3	Hamedan province: Malayer, Lashkardar protected area, Yousefi 19011.	34°12'45.14"N 48°58'46.25"E	2172	2n = 20	7 June 2011
4	Hamedan province: Nahavand, Sarabe Giyan, above farmland, Yousefi 19005.	34°08'33.86"N 48°13'03.64"E	1717	2n = 20	5 June 2011
5	Hamedan province: Nahavand, Sarabe Giyan, Yousefi 19006.	34°08'35.16"N 48°13'11.99"E	1698	2n = 20	5 June 2011
6	Hamedan province: Razan, Boghaty Mountains, Yousefi 19012.	35°02'19.5"N 48°50'18.1"E	2350	2n = 20	12 June 2011
7	Hamedan province: Tuyserkan to Malayer, Yousefi 19010.	34°28'25.57"N 48°33'03.63"E	1864	2n = 20	7 June 2011
8	Hamedan province: 5 km Nahavand to Malayer, left side of road, Yousefi 19007.	34°12'45.29"N 48°24'09.62"E	1775	2n = 20	5 June 2011
9	Hamedan province: Nahavand to Malayer, before Kartilabad village, right side of road, Yousefi 19009.	34°18'44.05"N 48°38'26.69"E	1697	2n = 20	5 June 2011
10	Hamedan province: Kabodarahang, around Gholiabad village, Yousefi 19014.	35°15'03.30"N 48°50'40.01"E	1906	2n = 20	12 June 2011

were pretreated using an aqueous solution of colchi (0.05%) at room temperature for 3 h. The material was fixed in absolute ethanol and glacial acetic acid (3:1) for 12 h at room temperature and then stored in the fixative at 4 °C. Samples were hydrolyzed in 1 N HCl for 2 min at 60 °C, stained in 1% aqueous aceto-orcein for 2–12 h at room temperature, squashed and mounted in a drop of 45% acetic acid-glycerol (9:1). The best metaphase plates were photographed with a Ziess Axiostar photomicroscope and a Canon digital camera. Karyotype analysis was carried out according to the method described by Levan et al. (1964). Several parameters regarding the karyotypes symmetry/asymmetry including total length of chromosome (C), length of long arm (L), length of short arm (S), arm ratio (R: L/S), centromeric index (I: (S/C)×100) and centromeric position were calculated for each accession with the use of the DN2 Microscopy Image Processing System. Morphometric data regarding karyotypes were provided and an ideogram of the species was depicted based on the average of several accessions karyotypes which have approximately the same level of chromosome condensation. To assess the existence of published chromosome counts in the studied species we used the chromosome number database Index to Plant Chromosome Numbers (Goldblatt and Johnson 1979).

Results and discussion

In the present paper, we determined chromosome numbers of *P. olivieri*. The chromosome numbers, karyotype analysis, and ideogram of *P. olivieri* are shown based on the

Tab. 2. Karyomorphological parameters of *Phlomis olivieri* based on the average values of ten accessions. SD – standard deviation, m – metacentric.

Chromosome no.	Total length (μm)±SD	Long arm length (L) μm	Short arm length (S) μm	Arm ratio L/S	Centromeric index	Chromosome type
1	7.67±0.68	4.09	3.58	1.14	46.67	m
2	7.12±0.82	3.73	3.39	1.10	47.61	m
3	7.11±0.82	3.87	3.24	1.19	45.56	m
4	6.99±0.72	3.81	3.18	1.19	45.49	m
5	6.74±0.73	3.71	3.03	1.22	44.95	m
6	6.64±0.73	3.56	3.08	1.15	46.38	m
7	6.52±0.65	3.65	2.87	1.27	44.01	m
8	6.31±0.72	3.44	2.87	1.19	45.48	m
9	6.20±0.61	3.43	2.77	1.23	44.67	m
10	6.06±0.73	3.39	2.67	1.26	44.05	m
11	6.03±0.69	3.35	2.68	1.25	44.44	m
12	5.86±0.67	3.25	2.61	1.24	44.53	m
13	5.73±0.69	3.07	2.66	1.15	46.42	m
14	5.54±0.48	2.93	2.61	1.12	47.11	m
15	5.48±0.55	3.10	2.38	1.30	43.43	m
16	5.27±0.53	2.97	2.30	1.29	43.64	m
17	5.29±0.52	2.94	2.35	1.25	44.42	m
18	5.10±0.52	2.83	2.27	1.24	44.50	m
19	4.87±0.78	2.83	2.04	1.38	41.88	m
20	4.69±0.74	2.61	2.08	1.25	44.36	m

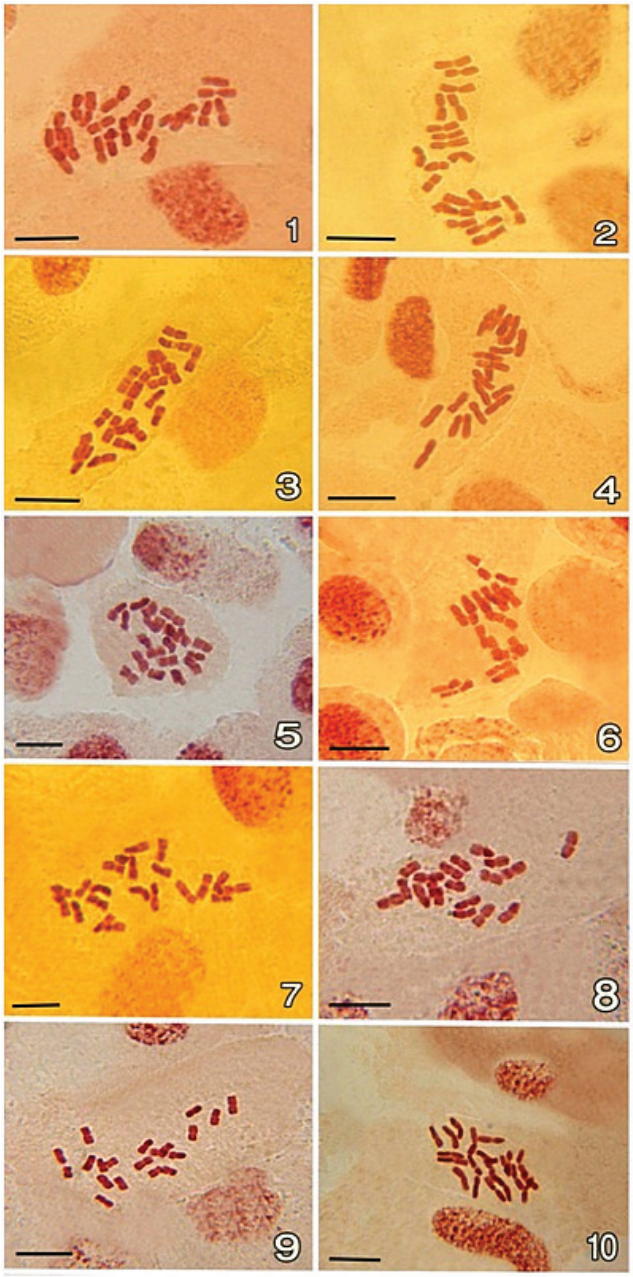

Fig. 1. Somatic metaphases in *Phlomis olivieri* (accessions 1–10) with diploid chromosome number 2n=20. Bar = 10 μm.

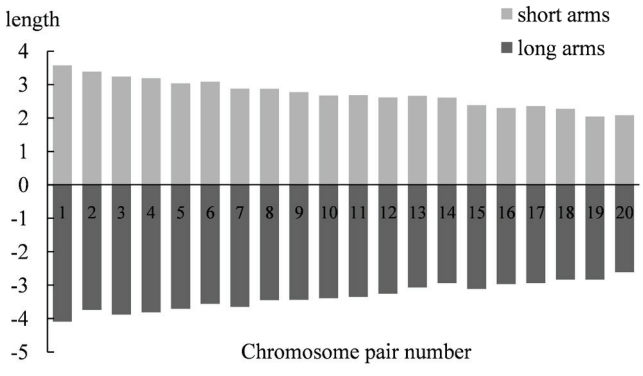

Fig. 2. Ideogram of *Phlomis olivieri*. Scale in μm.

average values of ten accessions (Tab. 1, Tab. 2, Fig. 1, and Fig. 2). This is the first karyotype determination for this species. Based on our results the basic chromosome number in the studied accessions was x=10. All accessions have the same chromosome number and represent diploid species with 2n=2x=20. Chromosome sizes vary from 2.66 to 8.60 μm. The longest arm is 4.796 μm (accession 2) and the shortest arm is 1.16 μm (accession 9) (Fig. 1.) Different karyotypes have been found among the accessions of *P. olivieri*. Generally, the studied karyotypes consist mostly of metacentric chromosomes with almost median centromere position (Stace 1989). However, the karyotypes of accessions 1, 4, 6, 7 and 9 beside metacentric chromosomes also possess submetacentric chromosomes. Such differences between accession karyotypes could be a result of different chromosome condensation due to the pretreatment procedure. This could be also a reason why secondary constrictions with satellites were not observed in the karyotype of this species. According to the average values of ten accessions, the karyotype of this species consists of 10 pairs of metacentric chromosomes (Tab. 2).

Azizian and Cutler (1982) revealed that species of the *Phlomis* section *Phlomis* are characterized by 2n=20 large chromosomes, while species of the *Phlomis* section *Phlomoides* possess 2n=22 and smaller chromosomes. Özdemir et al. (2014) determined 2n=20 chromosomes for *P. lunariifolia* Sm. and *P. grandiflora* H.S. Thomps., two species of the genus *Phlomis* sect. *Phlomis*. The investigated species had 9 pairs of metacentric and 1 pair of submetacentric chromosomes. Furthermore, the presence of 2n=20 chromosomes within the genus has been confirmed in several species such as *P. cypria* Post var. *cypria* (Yildiz and Gücel 2006), *P. lychnitis* L., *P. purpurea* L., *P. italica* L. and *P. herba-venti* L. var. *tomentosa* Boiss. (see Mateu 1986). Although it can be concluded that the presence of twenty somatic chromosomes in our study is consistent with the previous studies, karyotypic differences exist within or among species and alteration in chromosome symmetry may arise through translocations, pericentric inversions or fusion (Levin 2002).

Our observations as well as comparison of photomicrographs obtained from previous reports also showed that *Phlomis* chromosomes are the largest among species of the Lamiaceae genera such as *Callicarpa* L., *Salvia* L., *Scutellaria* L., *Sideritis* L., *Stachys* L., *Teucrium* L. and *Thymus* L. (Jalas 1948, Boşcaiu et al. 1998, Yildiz and Gücel 2006, Yang et al. 2009, Martin et al. 2011, Contreras and Ruter 2011, Javadi et al. 2011).

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