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Chromosome numbers and karyotypes of *Achillea oxyodonta* Boiss. and *Achillea aucheri* Boiss. (Asteraceae)

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Abstract

The cytological features including chromosome numbers and detailed chromosome measurements were reported in *Achillea oxyodonta* and *A. aucheri* from Iran. Total chromosome lengths, relative lengths, long/short arm ratios and centromeric index of mitotic chromosome were calculated. Somatic chromosome numbers were found to be 2n=2x=18 in *A. oxyodonta* and 2n=4x=36 in *A. aucheri* for the first time. The mean of chromosome length ranged from 4.00 to 6.40 µm in *A. oxyodonta* and from 3.66 to 6.33 µm in *A. aucheri*. The karyotype consists of seven pairs metacentric chromosomes and two pairs in *A. oxyodonta* and of fifteen pairs metacentric chromosomes and three submetacentric pairs in *Achillea aucheri*.

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Introduction

The genus *Achillea* L. (Asteraceae) comprising about110-140 perennial herb species (Saukel *et al.* 2004; Guo *et al.* 2004; Kiran *et al.* 2008) that are centered in SE Europe and SW Asia with extensions through Eurasia to North America.

A large number of species are endemic and restricted to certain regions, in contrast to other species from the genus growing over a wide geographical range (Azani *et al.* 2009).

Plants in this genus are perennial, entomophilous and predominantly outbreeding. The genus exhibits great ecological amplitude ranging from deserts to water-logged habitats and from sea coasts to the high mountains as a result of which, several *Achillea* species show high morphological variability (Ehrendorfer and Guo 2006).

The majority of the *Achillea* species are of medicinal values having therapeutic applications. The whole over ground parts and mainly the in florescences are effective as anti-inflammatory, spasmolytic, choleretic drugs (Boskovic *et al.* 2005) Essential oil and extracts of the plants are used for preparation of cosmetics, stomachic and digestive teas, creams, etc. Besides 1,8-cineole, compounds of bornane skeleton such as camphor and borneol are among the second and third most frequently characterized components of yarrow oil in *A. taygetea* and *A. fraasi* (Magiatis *et al.* 2002), *A. albicaulis* C. A. Mey. (Feizbakhs *et al.* 2003), *A. pseudoaleppica* Hub.-Mor. (Zen *et al.* 2003), *A. talagonica* Boiss. and *A. vermicularis* Trin(Rustaiyan *et al.* 1998). The basic chromosome number in *Achillea* is x = 9, wich polyploidy occurring frequently. The karyology of the genus has been studied by several researchers and reported chromosome numbers include 2n=18, 36, 54, and 72. (Oswiecimska 1974; Androschchuk and Kostinenko 1981; Magulaev 1982; Maffei *et al.* 1993).

Nineteen *Achillea* species are reported from Iran (Podlech, 1986) growing in different regions of the country that seven of them are endemic. Although *Achillea* species have been studied extensively in different regions of the world (Dabrowska 1977; Pireh and Tyrl 1980; Dabrowska 1989; Guo *et al.* 2004; Saukel *et al.* 2004; Guo *et al.* 2005; Ahmet 2006; Kiran *et al.* 2008; Sheidai *et al.* 20090, but no caryological works have been published on *A. oxyodonta* and *A. aucheri* as an endemic species in Iran. Therefore, the aim of this study is to determine the karyotypes and chromosome numbers of these two endemic species for the first time.

Materials and methods

Samples collection

A. oxyodonta and *A. aucheri* plants were collected from the natural populationms in Iran (Table 1). Seeds of these species were used in the study fo karyotyps analysis.

Chromosome counting

Karyotype - Mitotic chromosomes in root-tip cells were studied from squash technique. Seeds were germinated in darkness at 25 oC and them put in petri dishes on moist filter paper. Actively growing root tips 1 cm in length were excised from the germinating seeds; them were pretreated with 0.002 M 8-hydroxyquinoline for 24 hours at 25 °C and fixed in Carnoy (3:1 ethanol absolute: acetic acid) for 24 hours for at least 24 h at 4◦C, hydrolysed in 1N HCl at 60◦C for 15 min and then rinsed in tap water for a minimum of 3–5 min. Staining was carried out in 2 % orcein for 24 h and squash preparations were made with 45% acetic acid The slides were examined under a light microscope, and 10 well scattered metaphase plates were selected for karyotype analysis. Some of these metaphases were photographed and ordered in the karyotype on the basis of their length. For each chromosome, total length, short arm and long arm were measured and relative lengths, arm ratios and centromeric index were determined. Chromosome pairs were identified according nomenclature followed Levan *et al.*(1964).

Table 1. Locality of *Achillea* species

Species	Alt./m	Locality
Achillea oxyodonta	2500	Iran, North of Shemiran, Darband, Paseghale, Mazooji.
Achillea aucheri	4000	Iran, Tehran to Amol, Imamzadeh-e-Hashem, Mazooji, Salimpour.

Results

Achillea oxyodonta - had a chromosome number of 2n=2x=18 (Figs. 1a, 2a and 3). Karyotype formula, chromosome morphology, total and relative chromosome lengths, arm ratio and centromeric index were summarized in Table 2. At mitotic metaphase seven pair of metacentric (m-M) and two pair of Submetacentric (St) chromosomes were visible. The chromosome length varied from 4.00 to 6.40 µm. The relative chromosome length ranged from 8.26 to 13.22%. Total chromosome length was 48.40 µm.

Achillea aucheri - This species showed 2n=4x=36 chromosomes (Figs. 1b, 2b, 4). The chromosome number, chromosome morphology, total and relative chromosom lengths, arm ratio and centromric index of *Achillea aucheri* were detailed in Table 3. In the karyotype fifteen chromosome pairs were metacentric (m-M) and tree pairs Submetacentric (Sm). The chromosome length ranged from 3.66 to 6.33 µm. The relative length varied from 4.11 to 7.12%. Total chromosome length was 88.9 µm.

Table 2. Morphometric characteristics of the chromosomes of *A. oxyodonta*

Chromosome no	Chromosome length (µm)	Relative chromosome length (% of 2n)	Centromeric index	Arm ratio (long/short)	Chromosome morphology
1	6.40	13.22	46.00	1.13	m
2	6.20	12.80	48.00	1.06	m
3	6.00	12.39	50.00	1.00	M
4	5.80	11.98	44.00	1.23	m
5	5.40	11.15	48.00	1.07	m
6	5.20	10.74	30.00	2.25	Sm
7	4.80	9.91	45.00	1.18	m
8	4.60	9.50	34.00	1.87	Sm
9	4.00	8.26	45.00	1.22	m
T. C. L.	48.40				

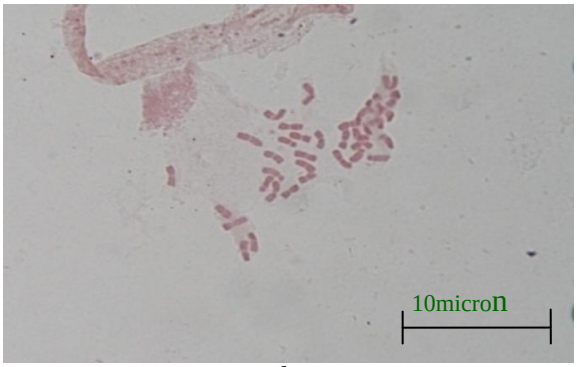
m-M= metacentric; Sm= Submetacentric; T. C. L.= Total chromosom length (haploid complement).

Table 3. Morphometric characteristics of the chromosomes of *A.aucheri*

Chromosome no	Chromosome length (µm)	Relative chromosome length (% of 2n)	Centromeric index	Arm ratio (long/short)	Chromosome morphology
1	6.33	7.12	47.00	1.11	m
2	6.33	7.12	31.00	2.16	Sm
3	6.00	6.74	50.00	1.00	M
4	5.66	6.36	46.00	1.12	m
5	5.50	6.18	36.00	1.75	Sm
6	5.49	6.17	48.00	1.06	m
7	4.99	5.61	46.00	1.14	m
8	4.83	5.43	48.00	1.07	m
9	4.83	5.43	41.00	1.41	m
10	4.83	5.43	37.00	1.63	m
11	4.66	5.24	42.00	1.33	m
12	4.66	5.24	28.00	2.50	Sm
13	4.33	4.87	46.00	1.16	m
14	4.33	4.87	42.00	1.36	m
15	4.32	4.85	38.00	1.60	m
16	4.16	4.67	39.00	1.50	m
17	3.99	4.48	41.00	1.40	m
18	3.66	4.11	45.00	1.20	m
T. C. L.	88.90				

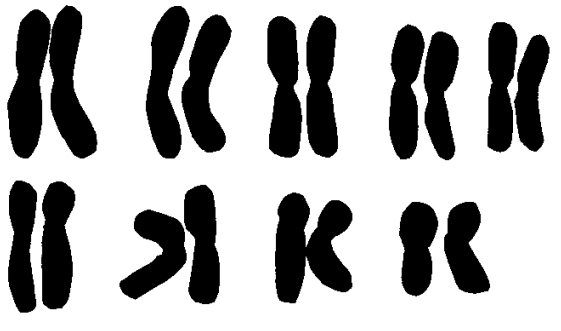
m-M= metacentric; Sm= Submetacentric; T. C. L.= Total chromosom length (haploid complement).

a

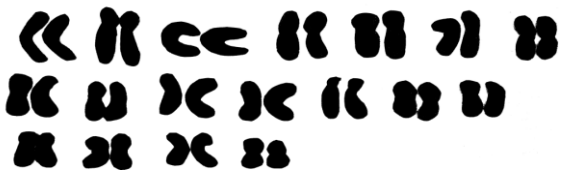


b

Fig. 1. Mitotic metaphase chromosomes of *Achillea* species. a. *Achillea oxyodonta* (2n=2x=18) b. *Achillea aucheri* (2n=4x=36). Scale bar= 10 µm



a



b

Fig. 2. Karyotypes of *Achillea* species. a. A. *oxyodonta,* b. A. *aucheri.*

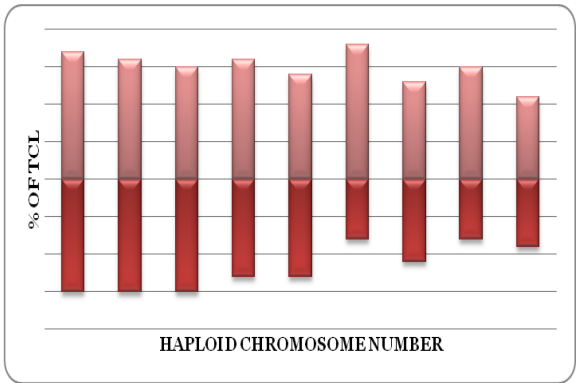


Fig. 3. Idiogram of *A. oxyodonta*.

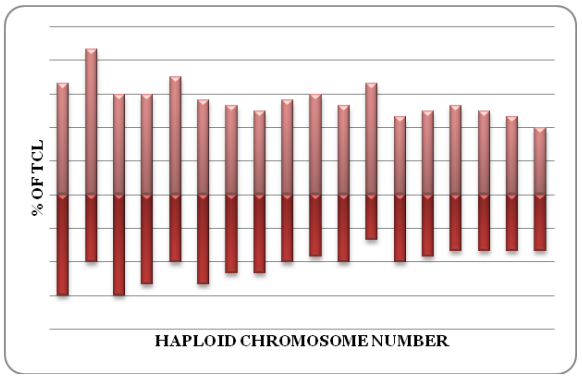


Fig. 4. Idiogram of *A. aucheri*.

Discussion

The genus *Achillea* is a polyploid complex; with basic chromosome number x=9, widely distributedin the Northern hemisphere. It is interesting because of its diversity, variability of some of its taxa and theexistence of problematic populations. The chromosome numbers of diploid *A. oxyodonta* (2n=2x=18) and tetraploid *A. aucheri* (2n=4x=36) were reported for the first time in this study, respectively.

Chromosome numbers of the species have been reported by several investigators. According these reports, *A. vermicularis* had 2n=18 (Dabrowska 1989; Sahin 2006) and 2n=36 (Khaniki 1995); *A. wilhelmsii* had 2n=18 (Khaniki, 1995); *A. millefolium* had 2n=18,27,36,54,72 (Ghaffari and Kelich 2006) ; *A. tenuifolia* had 2n=36 (Farsi *et al.* 2001) and 2n=27 (Khaniki, 1995). In all species, metacentric chromosomes are the most frequent followed by some submetacentric types.

Our cytological data for *A. oxyodonta* and *A. aucheri* consistent wich those of other investigators for *achillea* species in the number of chromosomes (2n=18, 36). Additionally, karyotypes are symmetrical wich complements composed of mainly metacentric chromosomes, as the others *Achillea* species.

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