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Chromosome counts in some *Alchemilla* species from north-east Anatolia

Sema Hayirlioglu-Ayaz & Hüseyin Inceer

Department of Biology, Faculty of Sciences and Arts, Karadeniz Technical University, 61080 Trabzon, Turkey; e-mail: sha@ktu.edu.tr

Abstract: This paper presents the results of karyological analysis of seven *Alchemilla* species collected from north-east Anatolia, Turkey, belonging to *Alchemilla* sect. *Alchemilla* subsect. *Heliodrosium* ser. Vulgares and subsect. *Calycanthum* ser. Elatae and ser. Calycinae. The following chromosome numbers were determined: *A. haraldi* $2n = 85$–105, *A. heterophylla* $2n = 85$–97, *A. hirtipedicellata* $2n = 86$–100, *A. oriturcica* $2n = 86$–102, *A. persica* $2n = 78$–99, *A. procerrima* $2n = 69$–78 and *A. trabzonica* $2n = 78$–88. The chromosome numbers of three of these seven species are presented for the first time.

Key words: chromosome numbers, polyploidy, Rosaceae, Turkey

Introduction

A critical and taxonomically difficult group, the genus *Alchemilla* L., which is distributed mainly in the Holarctic but occurs also in the mountains of East and South Africa as well as in those of Madagaskar, South India, Sri Lanka, and Java, comprises more than 1000 species (Izmailow, 1981; Fröhner, 1995).

In Turkey, the genus is, according to Pawlowski & Walters (1972), represented by 3 subsections (*Chirophyllum* Rothm., *Heliodrosium* Rothm., *Calycanthum* Rothm.) and 6 series (Saxatiles Bus., Sericeae Bus., Pubescentes Bus., Vulgares Bus., Elatae Rothm., Calycinae Bus.) all belonging to *Alchemilla* sect. *Alchemilla.* Most of them are found in north-east Anatolia.

Although the intricate taxonomy of *Alchemilla* has received much attention, cytological data for the genus are very scarce. Turesson (1957) determined the chromosome numbers for 19 species, Wegener (1967) for 56, Izmailow (1981, 1982) for 11, Hayirlioglu-Ayaz & Beyazoglu (1997a, 1997b, 1997c, 2000a, 2000b, 2001) for 49 and Hayirlioglu-Ayaz & Inceer (2001) for 5.

From the cytological and taxonomic point of view, *Alchemilla* presents a difficult subject. Izmailow (1981, 1982) reported that all studied species collected from the Western Carpathians (Poland) are high polyploids, and in spite of the small size of chromosomes, it is difficult to obtain countable metaphase plates to get exact chromosome counts. Diploid cytotypes have been unknown yet.

Already at the beginning of last century Strasburger (1905) discovered that many species of *Alchemilla* reproduce apomictically. The genus is the best known example of autonomous apomixis in Rosaceae. Apospory, parthenogenesis, advetitious embryoni, and apogamety occur comparatively frequent in the genus (Czapik, 1996). It is known that nearly all Eurasiatic species of the genus have proved to be apomictic; the pollen is wholly abortive and the seed develops precociously in the flower. The phylogeny of the genus in Europe and western Asia is not clear. The high chromosome numbers (Wegener, 1967) suggest complex hybrid origins of the recent species from sexual parentaly species which are now extinct, but in the development of species, separation and isolation must also be taken into consideration as highly important.

The present paper continues our previous karyological investigations, which involved 45 species of *Alchemilla* distributed in north-east Anatolia. Seven species were studied in the course of the present work.

Material and methods

Seven *Alchemilla* species (*A. haraldi* Juz., *A. hirtipedicellata* Pawl., *A. oriturcica* Pawl., *A. persica* Rothm. and *A. trabzonica* Hayirlioglu-Ayaz & Beyazoglu from ser. Elatae, *A. heterophylla* Rothm. from ser. Vulgares, and *A. procerrima* Fröhn. from ser. Calycinae) collected in the mountains of north-east Anatolia in June, July and August 1994, 1995 and 1999 were used in this study. Specimens were kept in the herbarium at Karadeniz Technical University Department of Biology (KTUB).

Root tip meristems obtained directly from natural populations were used for chromosome analysis. Young growing roots were cleaned of soil particles, their root tips were cut

Table 1. Somatic chromosome numbers ($2n$) of *Alchemilla* species investigated.

Species	Individual numbers	Metaphase cells counted	Chromosome numbers	$2n$
A. haraldi	20	13	85(5), 95–98(3), 100–102(2), 105(3)	85–105
A. heterophylla	22	14	85, 86(2), 90, 95–96(3), 96–97(3), 97(4)	85–97
A. hirtipedicellata	25	16	86(3), 86–87(4), 87–88(4), 88(2), 88–100, 100(2)	86–100
A. oriturcica	21	14	86, 93(3), 93–95(3), 94–95(2), 98–100(2), 101(3), 102	86–102
A. persica	23	15	78–80(2), 81(3), 85–87(4), 90–91, 94–96(3), 99(2)	78–99
A. procerrima	20	15	69, 69–70, 70(2), 70–71, 71(3), 76(2), 76–78, 78(4)	69–78
A. trabzonica	24	18	78(3), 78–80(4), 81,82–83(2), 85(2), 85–87(2), 87, 88(3)	78–88

off, pretreated with 0.5% colchicine for 3 hours and then fixed in an ethanol-acetic acid (3:1) solution for at least 24 h at 4°C (Hayirlioglu-Ayaz & Inceer, 2001). The root tips were hydrolyzed in 1 N HCl at 60°C for 15 min and then rinsed with tap water for 2–3 min. Stainning was carried out in Feulgen. Squashing was done in 45% acetic acid and the preparations were mounted in Entellan.

In view of the serious difficulties in karyological analysis of *Alchemilla*, the fixation of root tips was, as a rule, repeated several times for each specimen studied. For each species, 20–25 specimens were collected in the field. About 75–100 root tips were karyologically examined for each studied species. Fifteen permanent slides were prepared and at least 10 well-spread metaphase plates were photographed with an Olympus BH-2 camera and drawn from permanent slides deposited at the Department of Biology, Karadeniz Technical University, Trabzon.

Results and discussion

Alchemilla haraldi Juz.
A8 Rize: Ikizdere, above the village of Anzer, alpine meadows, stream banks, 2150 m a.s.l., 19.vii. 1995, Hayirlioglu-Ayaz *207*, KTUB.

So far, this species has not been reported from Turkey. The somatic chromosome number is $2n = 85$–105 (Tab. 1, Fig. 1). The chromosome number of this species has not been studied untill now.

Alchemilla heterophylla Rothm.
A7 Trabzon: Zigana Dağı, meadows, stream sides and margins of *Pinus sylvestris* forest, 1700 m a.s.l., 14. vii. 1994, Hayirlioglu-Ayaz *168*, KTUB.

The first chromosome count in this species was reported by Hayirlioglu-Ayaz & Beyazoglu (2001) as $2n = 100$–105. In this work, the chromosome number of this species was found to be $2n = 85$–97 (Tab. 1, Fig. 2).

Alchemilla hirtipedicellata Pawl.
A7 Trabzon: Zigana Dağı, meadows, stream banks, 1700 m a.s.l., 14.vii.1994, Hayirlioglu-Ayaz *164*, KTUB

In this work, the chromosome number of this species was found to be $2n = 86$–100 (Tab. 1, Fig. 3). Hayirlioglu-Ayaz & Beyazoglu (2000a) was the first to report the chromosome number of *A. hirtipedicellata* ($2n = 86$–96).

Alchemilla oriturcica Pawl.
A7 Trabzon: Zigana Dağı, meadows, stream banks, 1700 m a.s.l., 14.vii.1994, Hayirlioglu-Ayaz *165*, KTUB.

The first chromosome count in this species, which is endemic to Turkey, was reported by Hayirlioglu-Ayaz & Beyazoglu (2000a). They studied material from Çaykara, Soğanlı Dağı, and found the chromosome number $2n = 86$–106. In the present study, the chromosome number of *A. oriturcica* was determined as $2n = 86$–102 (Tab. 1, Fig. 4).

Alchemilla persica Rothm.
A8 Rize: Ikizdere, above the village of Anzer, open slopes, meadows, stream sides, 2150 m a.s.l., 9.vii.1995, Hayirlioglu-Ayaz *213*, KTUB.

Wegener (1967) was the first to report the chromosome number of *A. persica* ($2n = 101$–106). Later the chromosome number $2n = 96$–106 was reported by Hayirlioglu-Ayaz & Beyazoglu (2000a). In the present study, the chromosome number of *A. persica* was determined as $2n = 78$–99 (Tab. 1, Fig. 5).

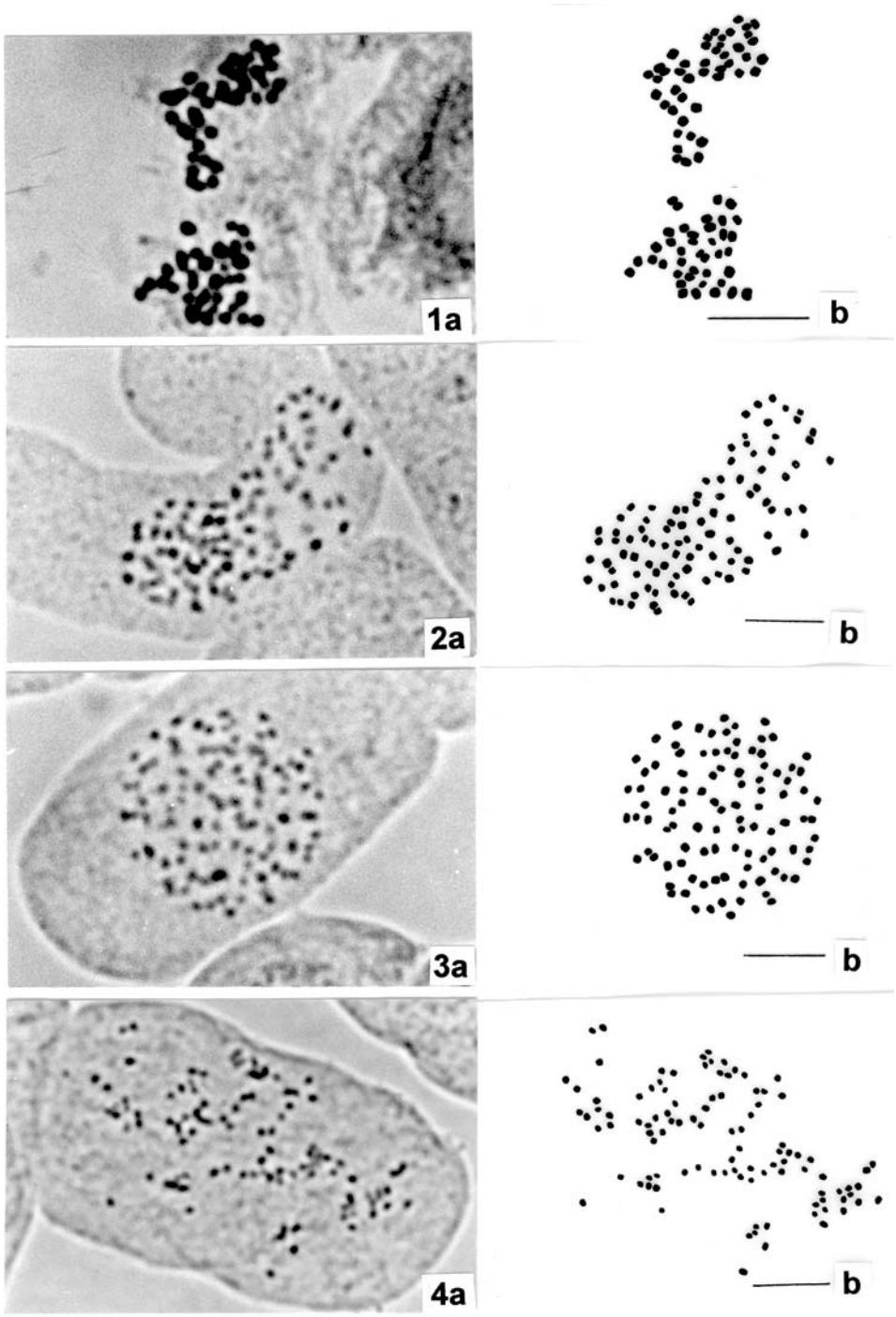
Alchemilla procerrima Fröhn.
A8 Rize: Ikizdere, above the village of Anzer, alpine meadows, stream banks, 2150 m a.s.l., 19.VII.1999, 2200 m, Hayirlioglu-Ayaz *208*, KTUB.

The chromosome number of *A. procerrima*, which is endemic to Turkey, is reported here for the first time, and the somatic chromosome number was $2n = 69$–78 (Tab. 1, Fig. 6).

Alchemilla trabzonica Hayirlioglu-Ayaz & Beyazoglu
A7 Trabzon: Zigana Dağı pass, meadows, stream side banks (together with some species of *Rhododendron*) 1750 m, 20.vi.1995, Hayirlioglu-Ayaz *190*, KTUB.

The chromosome number of *A. trabzonica*, which is endemic to Turkey, is reported for the first time. The somatic chromosome number was $2n = 78$–88 (Tab. 1, Fig. 7).

Former karyological studies of *Alchemilla* species belonging to section *Alchemilla* have revealed that they are represented by a series of very high polyploid cytotypes with the chromosome numbers ranging from 64 to ca. 224. About 75% of the species have chromosome

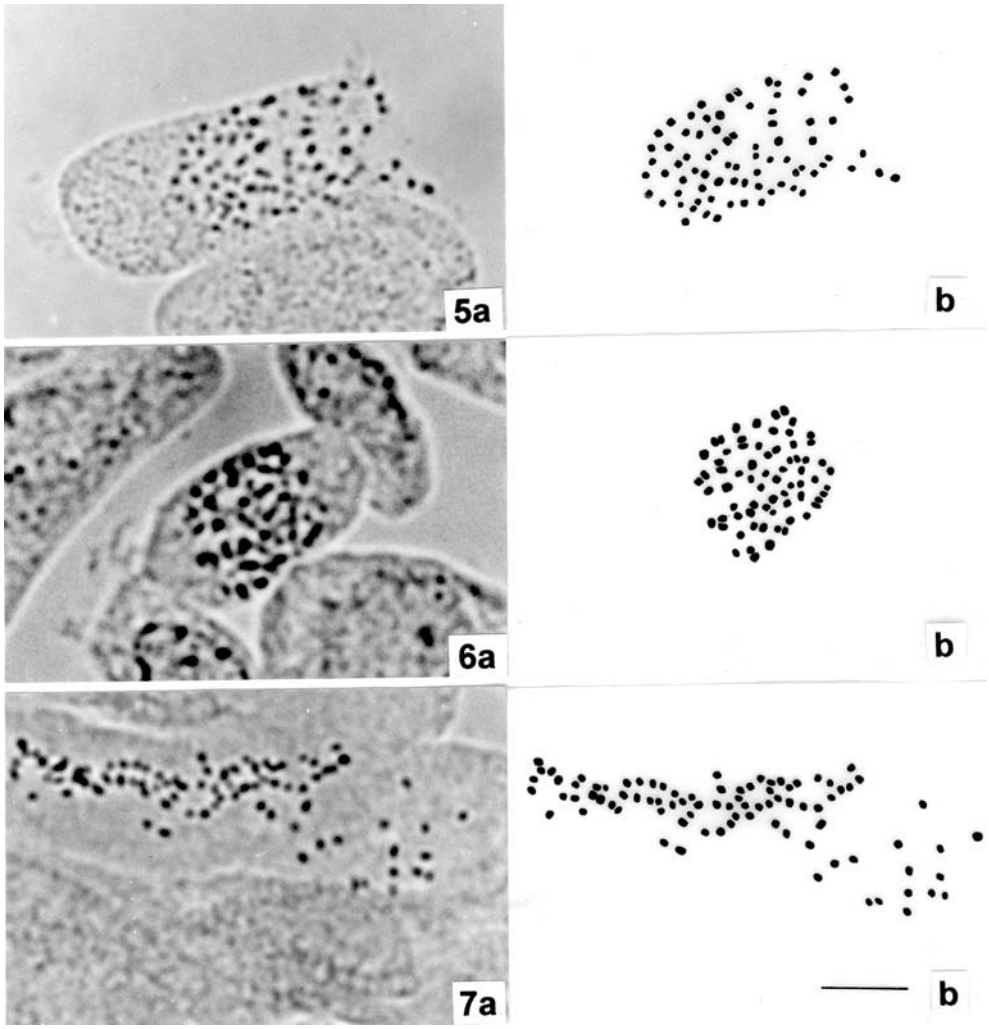


Figs. 1–4. Somatic metaphases (a: photograph, b: drawing). 1 – *Alchemilla haraldi* ($2n = 85$). 2 – *A. heterophylla* ($2n = 97$). 3 – *A. hirtipedicellata* ($2n = 100$). 4 – *A. oriturcica* ($2n = 101$). Scale bar 10 μm.

numbers of $2n = 96$–110 (Turesson, 1957; Wegener, 1967; Izmailow, 1981).

Comparison of data obtained by Wegener (1967) and Hayirlioglu-Ayaz & Beyazoglu (2000a, 2001) with the results of the present study shows that the specimens have different chromosome numbers. The diverging karyological results might result from intraspecific karyological differentiation. Such differentiation has been found in *A. alpina* ($2n = 137$–144 in Scandinavia and $2n = 119$–122 in the Alps, according to Turesson, 1958; $2n = 119$–129 in the central Alps according to Wegener, 1967). Similarly, Izmailow (1982) reported that specimens collected from various geographical regions have different chromosome numbers. Extensive karyological, molecular, and embryological studies might help explain the causes of karyological differentiation in *Alchemilla* species.

A very complex and hybrid origin of recent *Alchemilla* L. species is suggested also by their high and different chromosome numbers. Because of difficulties



Figs. 5–7. Somatic metaphases (a: photograph, b: drawing). 5 – *Alchemilla persica* ($2n = 78$). 6 – *A. procerrima* ($2n = 69$). 7 – *A. trabzonica* ($2n = 87$). Scale bar 10 μm.

in determining the exact chromosome numbers as well as basic numbers and karyograms in *Alchemilla*, at present these features are not taken into consideration as additional criteria in the elucidation of highly complicated taxonomical and phylogenetic problems in *Alchemilla* (Izmailow, 1982). Gentscheff & Gustafsson (1940) and Gudjonsson (1941) suggested that $x = 7$ is the common basic number in Rosoideae. Later, Löve & Löve (1948) and Raven (1975) have suggested that the basic number in *Alchemilla* is $x = 8$. So far, our continual investigations on determining the chromosome counts of *Alchemilla* species have revealed that 66% of the species were represented $x = 7$ and the rest was $x = 8$ (34%). Noticeably, it cannot be ignored that aneuploidy is common in the genus. In the light of the karyological aspects during past decade, the genus should be considered as $x = 7$ and rest $x = 8$.

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