Cytogenetics of some of the Turkish drogs

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Abstract

The karyotype analyses of *Cannabis sativa* L., *Carthamus tinctorius* L., *Coriandrum sativum* L., *Cuminum cyminum* L. and *Medicago sativa* L. used as folk medicine in Turkey are conducted using Image Analysis System. The somatic chromosome numbers of these species are determined respectively as follows; in *Cannabis sativa* $2n = 20$, in *Carthamus tinctorius* as $2n = 24$, in *Coriandrum sativum* as $2n = 22$, in *Cuminum cyminum* as $2n = 14$ and in *Medicago sativa* as $2n = 32$. The karyotype analyses revealed in *Carthamus tinctorius* three pairs and in *Cuminum cyminum* two pairs of mitotic metaphase chromosomes with satellites. Tetraploidy was seen only in the species *Cuminum cyminum* and *Medicago sativa*. In the species of *Cannabis sativa* one B chromosome was determined. The idiograms of the species have been conducted as well.

Key words: *Cannabis*, *Carthamus*, *Coriandrum*, *Cuminum*, *Medicago*, Karyotype.

INTRODUCTION

Humanity relies on a diverse range of cultivated species; at least 6000 such species are used for a variety of purposes. It is often stated that only a few staple crops produce the majority of the food supply. This might be correct but the important contribution of many minor species should not be underestimated. Agricultural research has traditionally focused on these staples, while relatively little attention has been given to minor (or underutilized or neglected) crops, particularly by scientists in developed countries. Such crops have, therefore, generally failed to attract significant research funding [1].

Cannabis sativa has been the source of a medicinal drug for centuries. In recent years there has been a renewed interest to reintroduce the drug into medicine. Properties attributed to it include analgesic–hypnotic; antiepileptic–antispasmodic; appetite stimulant; prophylactic and treatment of the neuralgia's, including migraine; anti-depressant–tranquilizer; psychotherapeutic aid; antiasthmatic oxytoxic; antitussive; topical anesthetic; withdrawal agent for opiate alcohol addition; childbirth analgesia; and even an antibiotic [2]. Drug varieties, previously described, are numerous. Another product from *Cannabis* is the fruit (achenes or seeds). They contain oil that is similar to linseed oil and therefore used in making soap and paints. The pressed cake resulting from oil extraction is fed to cattle. Bird seed mixtures often contain *Cannabis* seeds. Roasted achenes are eaten by some Europeans. Finally, the seeds are used as a culture medium in microbiology laboratories for water molds. The antibiotics extracted from these molds are active against gram-positive bacteria [3].

Safflower, *Carthamus tinctorius* L., is a member of the family Compositae or Asteraceae, cultivated mainly for its seed, which is used as edible oil and as birdseed. Traditionally, the crop was grown for its flowers, used for colouring and flavouring foods and making dyes, especially before cheaper aniline dyes became available, and in medicines [1]. Oil has been produced commercially and for export for about 50 years, first as an oil source for the paint industry, now for its edible oil for cooking, margarine and salad oil.

In China, safflower is grown almost exclusively for its flowers, which are used in treatment of many illnesses as well as in tonic tea. Safflower has a bitter herbal taste, but the Institute of Botany of the Chinese Academy of Sciences in Beijing has developed a nonbitter, sweet-smelling tea which contains amino acids, minerals and vitamins B1, B2, B12, C and E. Safflower preparations should be stored in light-resistant containers [4]. The main active ingredient in safflower medicines is safflower yellow, which is water-soluble, but alcohol extracts are used in some preparations. Many clinical and laboratory studies support the use of safflower medicines for menstrual problems, cardiovascular disease and pain and swelling associated with trauma.

Coriandrum sativum L. coriander is used as vegetable and spice [5] but the main part that is being used is its fruit. The fruits are used as spices in its natural form or in processed forms as essential oil in food, beverage and perfumery industry [6-8]. The petroselenic acid, to be found naturally only in the Umbelliferae family members, is in coriander between 60-70% [9]. Due to their antimicrobial effects, petroselenic acids are used widely in perfume and food industries [10]. In folk medicine it has been used for ages because of its appetizing, flatulent, and digesting characteristics [6, 7, 11]. *Cuminum cyminum* L. fruits have solid and essential oils, seeds and resins. It is used as appetizer, flatulent, and for perspiration.

Cumin is used as a medical plant and as a spice since ancient times. It is used in alternative medicine as flatulent, spasmodic, and headache-relief. Furthermore, it is an important

Ferro-source and relieves the digestive system as well. The essential oil of cumin-oil is effective of on the gastro-intestinal system. It has got an antimicrobial and a strong anti-toxic effect. It is used in cosmetics as well. It has a vitalizing effect upon the metabolism. It is used for weight loss, as a uretic and to increase mother milk and sexual performance. It is useful in treating hypertension and neural based dizziness. It dries up the moistness of the body, thins the blood, and removes the inertia from the toes and fingers. It dissolves the stones in the bladder and enables urination.

Medicago sativa L. is and important food for cattle. It has got like other animal food in its roots proteo-bacteria like *Rhizobia* increasing the nitrogen values in the plants. These bacteria provide a foodstuff with high protein regardless of the nitrogen amount in the soil. It makes the symptoms before menstruation milder, hinders blooding, strengthens systole, cleans the blood, and enables urination. Increases muscle mass, relax them, hinders anemia, strengthens bones and supports treatment. Strengthens the veins and the capillaries.

The aim of the present study is to determine the chromosome morphologies of five widely used species in folk medicine. With this aim, the karyotype analyses are made for the first time (except *Medicago sativa*) using Image Analysis System.

MATERIAL AND METHODS

The mature seeds of these five species are purchased from the attars in Konya. After the seeds have been cleaned they have been germinated in room temperature. In order to enable germinating easily the seed surfaces have been rubbed. The mitotic metaphase chromosomes are investigated in the root tips of the seeds. When the root tips reached 1-1.5 cm they are pretreated in α-monobromonaphthalene for 16 hours at +4Cº. After this first procedure, the root tips are fixed in 3:1 alcohol acetic acid for 24 hours and then placed into 70% ethyl alcohol for storage. The root tips, cleaned from alcohol with purified water, are hydrolyzed cold. After hydrolyzed, all species are stained with 2% aceto-orcein for two hours. The procedure for making them permanent has been conducted with liquid nitrogen. Depex has been used for covering. Permanent slides containing the chromosomes at the metaphase stage during the mitosis to make the karyotype analyses, measure the chromosome length and to count the chromosomes of each taxon. After the determination of the root tip somatic cells, distributed well in the preparations, having clearly observable morphologies and within the same focus, they are photographed with a camera attached to the microscope enlarged 10x100. The procedures to determine the centromeric position, arm ratio, chromosome arms and total length are conducted with the BsPro200 Image Analysis System after the transfer of the images to the computer.

RESULTS

Chromosome morphologies, total chromosome lengths, arm ratios and centromeric indices were summarized in Table 1. The chromosome number in *Cannabis sativa* as $2n = 20$, in *Carthamus tinctorius* as $2n = 24$, in *Coriandrum sativum* as $2n = 22$, in *Cuminum cyminum* as $2n = 14$ and in *Medicago sativa* as $2n = 32$ was counted for species. The results for somatic chromosomes are described below.

***Cannabis sativa* L.**

This species showed $2n = 2x = 20 = 7m+3sm$ Fig1. with a basic chromosome number of $x = 10$. The metaphase karyotype consisted of 7 metacentric chromosomes plus 3 submetacentric chromosome. The species had one B chromosome. Chromosomes are gradual from 0.76 µm to 1.39 µm. Total haploid chromosome length is 10.62 µm. The idiogram was given Fig6.

***Carthamus tinctorius* L.**

This species showed $2n = 2x = 24 = 9m+3sm$ Fig2. with a basic chromosome number of $x = 12$. The metaphase karyotype consisted of 9 metacentric chromosomes plus 3 submetacentric chromosome. The species had three satellite metaphase chromosomes. Chromosomes are gradual from 0.85 µm to 1.80 µm. Total haploid chromosome length is 15.84 µm. The idiogram was given Fig6.

***Coriandrum sativum* L.**

This species showed $2n = 2x = 22 = 4m+5sm+2ac$ Fig3. with a basic chromosome number of $x = 11$. The metaphase karyotype consisted of 4 metacentric chromosomes, 5 submetacentric plus 2 acrocentric chromosome. Chromosomes are gradual from 1.14 µm to 1.88 µm. Total haploid chromosome length is 16.48 µm. The idiogram was given Fig6.

***Cuminum cyminum* L.**

This species showed $2n = 2x = 14 = 2m+5sm$ Fig4. with a basic chromosome number of $x = 7$. The metaphase karyotype consisted of 2 metacentric chromosomes plus 5 submetacentric chromosome. The species had double satellite metaphase chromosomes. The species had tetraploid cells. Chromosomes

Table 1. AR: arm ratio; CI: centromeric index; THC: total length of haploid complement; M: metacentric; SM: submetacentric; AC: acrocentric; X: chromosome basic number, * pair of satellites is shown in the chromosome pairs are marked with an asteriks.

Taxon	2*n*	Ploidy level	Chromosome sizes (µm)	AR	CI	THC (µm)	M	SM	AC
Cannabis sativa	20	-	0.76-1.39	1.50	4.08	10.62	7	3	-
*Carthamus tinctorius**	24	-	0.85-1.80	1.42	3.47	15.84	9	3	-
Coriandrum sativum	22	-	1.14-1.88	1.99	3.11	16.48	4	5	2
*Cuminum cyminum**	14	4*x*	1.44-2.46	2.02	4.83	12.74	2	5	-
Medicago sativa	32	4*x*	0.92-1.78	1.42	2.60	19.48	15	1	-

are gradual from 1.44 µm to 2.46 µm. Total haploid chromosome length is 12.74 µm. The idiogram was given Fig6.

***Medicago sativa* L.**

This species showed $2n = 2x = 32 = 15m+1sm$ Fig5. with a basic chromosome number of $x = 8$. The metaphase karyotype consisted of 15 metacentric chromosomes plus 1 submetacentric chromosome. The species had tetraploid cells. Chromosomes are gradual from 0.92 µm to 1.78 µm. Total haploid chromosome length is 19.48 µm. The idiogram was given Fig6.

DISCUSSION

The chromosomal numbers of five species were determined based on an analysis of somatic metaphases. According to these data, a basic chromosome number of $x = 7$, 8, 10, 11 and 12 were established for this species. The total haploid chromosome lengths ranged from 10.62 to 19.48 µm with average chromosome lengths from 0.76 to 2.46 µm. The metacentric (m) and submetacentric (sm) chromosomes are found to form the main part of chromosome complement while the acrocentric (ac) chromosomes were rare or absent. Tetraploidy ($2n = 4x = 32$) is reported in *Cuminum cyminum*

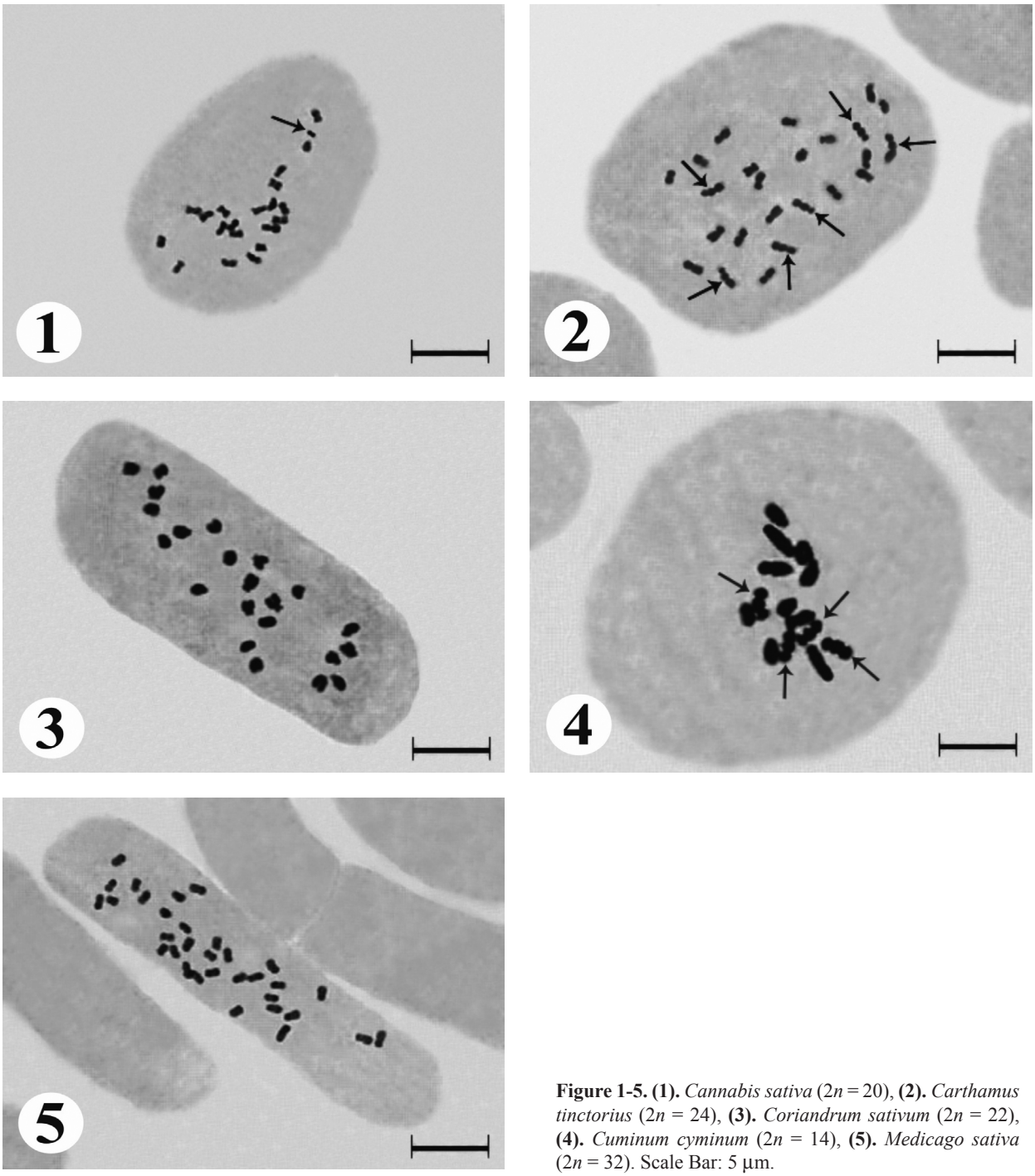

Figure 1-5. (1). *Cannabis sativa* ($2n = 20$), **(2).** *Carthamus tinctorius* ($2n = 24$), **(3).** *Coriandrum sativum* ($2n = 22$), **(4).** *Cuminum cyminum* ($2n = 14$), **(5).** *Medicago sativa* ($2n = 32$). Scale Bar: 5 µm.

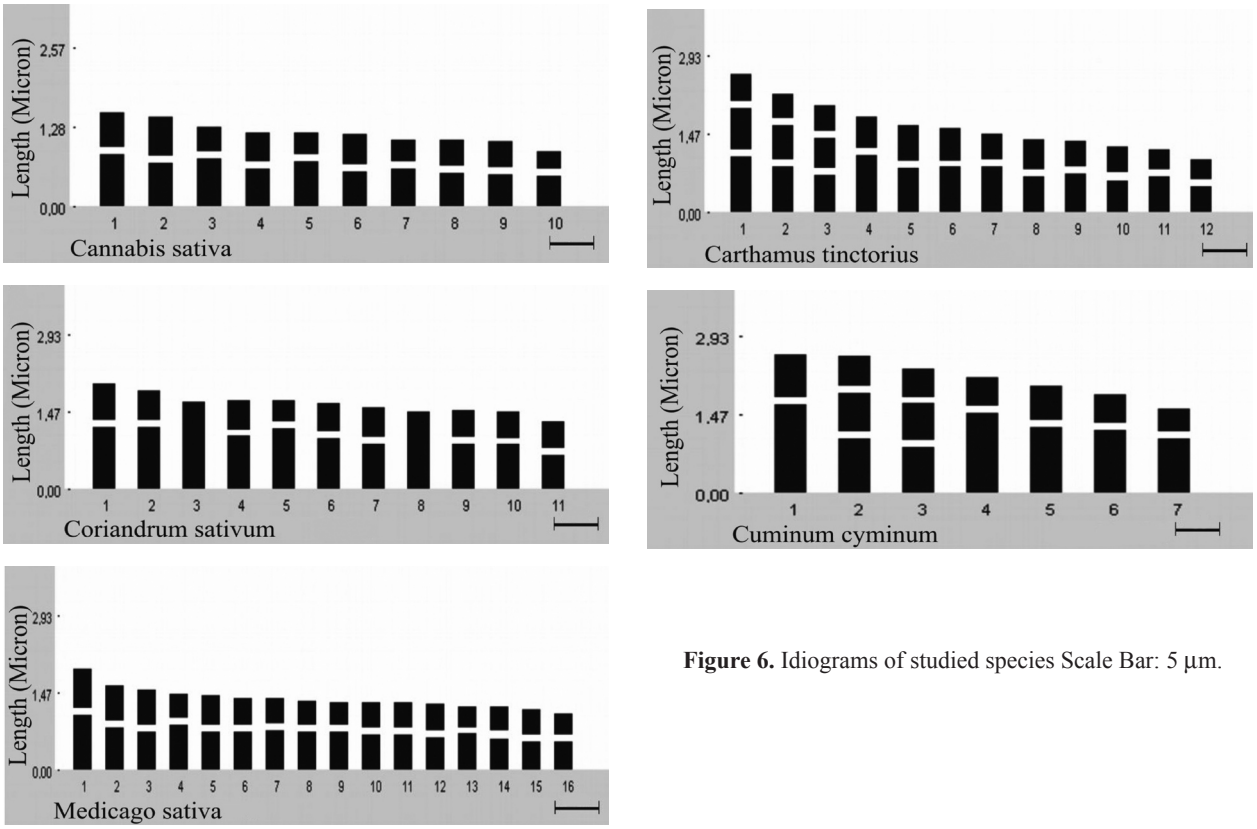

Figure 6. Idiograms of studied species Scale Bar: 5 µm.

and *Medicago sativa*. SAT chromosomes were analysed for two species (*Carthamus tinctorius* and *Cuminum cyminum*). Among the species analyzed in *Cannabis sativa* one B chromosome was observed.

The diploid chromosome number of *Cannabis sativa* is $2n = 20$. One B chromosome was determined in this species as well. Among the species studies, the shortest chromosome length is (0.76 µm) and the total haploid chromosome length is (10.62 µm). The chromosome number of *Cannabis sativa* is $2n = 20$ [12]. The somatic chromosome number of this taxon is similar to that given in literature.

Our analyses determined the diploid chromosome number of *Carthamus tinctorius* as $2n = 24$, with three pairs of double satellite mitotic metaphase chromosome. It has together with *Medicago sativa* the smallest arm length (1.42 µm) among the species studied. In the literature the somatic chromosome number of the taxa of genus C*arthamus* as $2n = 20$, 22, 24, 44 and 64 [13]. Our karyological findings are parallel to the literature. Three different basic chromosome numbers of this genus are mentioned as $x = 10$, 11 and 12 [14]. As the diploid chromosome number of *C. tinctorius* in our study is $2n = 24$ it is among those with a basic chromosome number of $x = 12$.

Coriandrum sativum's diploid chromosome number is $2n = 22$. Only this species has two double acrocentric the chromosome pairs. The basic chromosome number is $x = 11$ [15]. In the cytogenetical studies of the plants belonging to the Umbelliferae family in India by Sharma & Ghosh in 1955, the somatic chromosome number of the species *Coriandrum sativum* was reported as $2n = 22$, like in our study.

The diploid chromosome number of *Cuminum cyminum* species is $2n = 14$ and has a double satellite mitotic metaphase chromosome. This species has the longest chromosome length (2.46 µm), arm ratio (2.02) and centrometric index of (4.83). As in *Medicago sativa* it has tetraploid cells. Sharma & Ghosh in 1955, reported in their cytogenetical study of the plants in the Umbelliferae family in India the somatic chromosome number of *Cuminum cyminum* as $2n = 14$ [15]. This finding is identical to our finding in the present study.

The diploid chromosome number of *Medicago sativa* is $2n = 32$, with tetraploid cells with $2n = 4x = 64$ observed. This species has the shortest arm ratio among the species studied (1.42 µm) and a centrometric index of (2.60). Moreover, this specie has the longest total haploid chromosome length of (19.48 µm). Numerous karyological studies have been performed on alfalfa (*Medicago sativa* L. $2n = 4x = 32$), but only a few have examined the somatic chromosomes [16]. This condition is parallel to our study.

In 2001, Bauchan and Campbell have determined tetraploidy in their karyological study of species *Medicago sativa* [17]. The somatic chromosome number as determined by them is in our study $2n = 4x = 32$ as well. They too have determined the karyotype of this species using an Image Analysis System like the present study. Besides, they measured the chromosome lengths as between 2 µm and 3 µm. In the present study the chromosome morphology of this species is similar to that in the literature as the metaphase chromosome ranges between 0.92 µm and 1.78 µm.

In this study, the chromosome morphologies of five species, used as drugs in Turkey, are determined.

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