Determining Chromosome Numbers Of *Papaver* Species Grown In The Natural Flora Of Turkey

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***Abstract*— This study was conducted to determine chromosome numbers of wild *Papaver* species in the *Oxytona* section belong to the *Papaveraceae* family. Accessions were collected from Fesleğen Village/Niğde, Yıldız Mountain/Sivas, Tecer Mountain/Sivas, Ovacık Mercan Valley/Tunceli and samples gathered from Ankara University were used as materials in the study. Chromosome counting was performed on 89 accessions representing five regions in where wild papaver accessions grow naturally. Root tips were pretreated with 0,003 M alfa-bromonaphtaline solution and then fixed in the Carnoy's First Solution (3:1, Ethanol:Glacial Acetic Acid). Later, root tips were hydrolized in 1 N and then samples were stained in 2 % Aceto-Carmine dye. Chromosome numbers of all accessions were determined by counting chromosomes of the cells with complete and well distributed mitotic chromosomes. Based on the results of the study a ploidy series from diploid (2*n*=14) to hexaploid (2*n*=42) have been determined in the collection investigated. However, only four of the accessions were diploid which were identified as *P. bracteatum* while majority of the accessions were polyploid. In addition, karyotype analysis was performed in 3 species. All of the tetraploid accessions (2*n*=28) were identified as *P. orientale* while hexaploids were identified as *P. pseudo-orientale* (2*n*=42).**

Keywords— Chromosome numbers; cytology; *taxonomy; papaveraceae*

I. Introduction

There are about 200 species of *Papaver* L. genus around the world [1]. The general characteristics of the family are annual or perennial herbaceous plants, and they are in the form of tree sorshrubs.The Oxytona section belongs to the *Papaver* genus and includes perennial species. Its hows natural distribution in Central and Eastern Turkey, North and North West Iran, Caucasus and Trans-Caucasian regions.

Papaver bracteatum belonging to the *Oxytona* section, *P. orientale* and *P. pseudo-orientale* are morphologically similar to each other, but should not be confused with other papaver species. All three species are perennial and form rosettes every year, with pinnate and toothed leaves. The petiole of the main leaves is long and the entire leaf is covered with multicellular white hairs [2]. However, some plant characteristics, alkaloid contents and chromosome numbers are different [3].

Boissier [4], in his book 'Flora Orientalis', reported that 23 *Papaver* species, 11 perennial and 12 annual, grow in Turkey. In the following years, Fedde's monograph on the *Papaveraceae* family reported that there are 38 *Papaver* species, 21 perennial and 17 annual, in Turkey [5]. Davis [6] stated that there are a total of 39 *Papaver* species, 19 annual and 20 perennial (two subspecies and seven varieties), in Flora of Turkey, ten species, two subspecies, and four varieties are endemic to the flora of Turkey.

The *Papaveracea* family of the plant kingdom has six genus, including *Chelidonium, Glaucium, Hypecoum, Meconopsis, Papaver* and *Roemeria*. Among them, the genus *Papaver* L. with its around 100 species is the most important one in human nutrition and health [7,8]. The genus *Papaver* is distributed all over the World but mainly in Asia and Europe [8,9]. The genus was reported to comprise 9 sections [5] and then later was subdivided to 11 sections [10]. Over the years, several different types of *Papaver* were identified as annual, biennial or perennial species [7]. Although most of its annual species are known as weeds around the fields, its cultivated varieties are economically and medically very important for pharmacological products. *Papaver* L. were determined to be the source of one or more alkaloids over 170 alkaloids from 13 alkaloid groups [1,11]. Five alkaloids, namely morphine, codeine, the baine, papaverin and narcotine have major pharmacological significance [12].

Chemical, cytological, anatomical, embryological, palynological, physiological and so on characters are used in modern taxonomy as well as morphological characters used in classical taxonomy. Cytological

characters are usually related to chromosomes and mainly to chromosome numbers [13]. The basic chromosome numbers in the genus *Papaver* were determined to be x=6 which can be found in the diploid level only, x=7 which was observed in diploid, tetraploid, hexaploid, octoploid and decaploid levels and x=11 which was found in diploid and tetraploid levels [9]. If the chromosome observations done correctly, number of chromosomes is very useful characters. However, satellites are like a real chromosome, which can lead to an in correct chromosome count. This situation must be taken into consideration [14, 15]. Karyotype analysis should be made to determine all of these characters [16-20]. Karyotype analysis are basic cytological studies providing useful information for plant breeding and identifying genotypes [21]. A karyotype can be determined by comparing five different characters: differences in sets of chromosome size, centromere position, the relative sizes of chromosomes, the basic chromosome number and the satellite position and number [22]. In *Oxytana* section, somatic chromosome number was determined to be 2n=14 in *P. bracteatum*, 2n=28 in *P. orientale* and 2n=42 in *P. pseudo-orientale* [2, 23, 24].

The aim of this study was to determine chromosome numbers of three species of the section of *Oxytana* grown naturally in Turkey by using karyotype analysis.

II. MATERIAL and METHOD

Seeds of 89 accessions from the section *Oxytona of Papaver* L. obtained from different regions of Turkey (Yıldız and Tecer Mountains/Sivas, Ovacık Mercan Valley/Tunceli, Fesleğen Village/Niğde, Ankara University) were used in the study (Table 1).

A. Germination of Seeds

Approximately 30-35 seed grains were left in each petri dish. Distilled water was poured into the petri dishes to initiate germination, and the petri dishes were wrapped and covered with a film and kept under light for 10 hours in order to germinate under optimum conditions. No surface sterilization process was applied to the seeds. However, no signs of disease were observed in the germination medium.

B. Chromosome fixing

The tissue material used for the observation of somatic chromosomes was obtained from the root tips. When the root tips reached a sufficient length in petri dishes, they were removed from the environment and subjected to the process of stopping cell division, which is the first step in somatic chromosome monitoring. For this purpose, the α-bromonaphthalene method was used.

In this method, after several trials for papaver seeds used, when 1000 ml of α-bromonapthalene solution was kept at a level of 0.003 M, it was observed that somatic chromosomes were observed much more clearly in metaphase during cell division.

To be used in the study, 100 ml solutions were prepared as stock solutions and stored in small dark bottles. α-bromonapthalene placed in distilled water was mixed with a magnetic tirrer in a beaker for 30 minutes. After all these processes, α-bromonapthalene chemical was prepared and it was taken into dark colored bottles and kept in a dark environment.

Germinated seeds were taken into existing mall tubes and kept in the prepared α-bromonapthalene chemical for 3 hours at room temperature. At this point, temperature is of great importance. If the temperature is much higher than room temperature, the probability of somatic chromosomes to be seen in metaphase will decrease, as the adhesion of the chromosomes to each other will increase and it will be very difficult to separate them.

For the fixation of somatic chromosomes, 3:1 Ethanol: Acedic Acid Solution, called Carnoy's 1st solution, was used. Carolan et al. [25] method, 3 units of ethyl alcohol (absolute alcohol -C2H5- OH) and 1 unit of glacial acetic acid (CH3-COOH) are used. At this stage, following the extraction of the root tips, the root tips are kept in the prepared 3:1 fixative for two days, and the chromosomes are cleared from unwanted tissues and cells and exposed. The fixation process was applied on the condition that the root tips taken into eppendorf tubes were kept at room temperature for two days. Thus, we tried to fix the cell divisions that we stopped in this second step, which we applied by stopping the cell divisions at the metaphase stage in the first step. After two days of waiting, the hydrolysis process was started.

In 1 N HCL used in the hydrolysis process, timing and temperature are important for the breakdown of purine-dioxyribose bonds in the tissues and cells we mentioned [26]. For this, the root tips removed from the stabilizer were rinsed thoroughly with distilled water, then dried with blotting papers and kept in 1 N (HCL) at 60 C^0 for 8 minutes [25]. In the study, Aceto-Carmine dye, which was effective from these dyeing methods, was used. 100 ml of distilled water and 45 ml of Glacial Acedic Acid were mixed and heated until boiling in a fume hood. 2 g of Carmine Powder was weighed on a precision balance and mixed into boiling distilled water and Glacial Acedic Acid solution with the help of a magnetic stirrer. After a mixing process of about 10 minutes, the dye solution was taken from the fume hood to the outside, left to cool, and then filtered. Whatman 1 papers were used in the filtering process. Afterwards, the filtered dye was taken into dark colored bottles and stored at +4 °C. After hydrolysis, the root tips were washed in distilled water and cleaned, then left on the blotter for a few seconds, dried and taken into the dye. The samples taken into the dye were labeled and stored in a dark compartment.

The root tips were squash edusing acetic acid squash technique and prepared slides were observed under a NikonEclipse E 600 microscope and good

looking cells were photographed by NikonCoolpix 5400 camera installed on the microscope.

C. Karyotype analysis and idiogram

In the study, the ploidy levels of the species belonging to the Oxytona section of the *Papaveraceae* family were found as *P. bracteatum* diploid, *P. orientale* tetraploid and *P. pseudo-orientale* hexaploid. While performing karyotype analysis, karyotype analysis was performed based on ploidy levels in n chromosome number (haploid). The genomes of the species are named in the n chromosome structure (haploid). In this case, *P. bracteatum* (A), It had the genome structure of *P. orientale* (AB) and *P. pseudo-orientale* (ABC). In other words, the chromosomes of all there species exist in similar proportions in their genome structures. Homologous chromosomes in genomes during karyotype analysis of species, Lavania and Srivastava [17] on *P. bracteatum* based on the data in the karyotype analysis study.

Photographs taken during the study were used for karyotype analysis. These photos contain the chromosome numbers of all three species. The photographs taken were enlarged on A4 paper size and homologous chromosomes were matched by giving numbers to each chromosome. The centromere regions of the chromosome groups with in the genomes of the species were found by aligning the long arm to the short arm ratio. In this ratio, arm lengths of chromosomes in a single genome in *P. bracteatum* haploid structure were calculated and matched. Chromosome pairs of A and B genomes in haploid structure were calculated and matched in *P. orientale.* In *P. pseudo-orientale,* chromosome pairs of A, B and C genome structures in haploid structure were calculated and matched.

While making the idiograms, the ratios of 7 chromosomes in the n chromosome (haploid) structure were arranged from the largest to the smallest. In this order, the centromere regions of the chromosomes are left blank with a length of 2 mm. The arms are aligned in the same plane.

III. RESULTS and CONCLUSIONS

The seeds were germinated in plastic petri dishes. Germination rates ranged from 10% to 95%. Germination times varied between 7-10 days according to the samples. This difference is due to the fact that plants are wild species and have physiological differences. Therefore, seeds with proper germinations were used in there search.

Good looking mitotic chromosomes of 89 accessions were photographed as the one shown in Figure 1. When we look at the distribution based on chromosome numbers, it can be concluded that *P.orientale* and *P. pseudo-orientale* species are found more common than *P.bracteatum* species in nature. According to results, 43 of 89 plants were hexaploid with 2n=6x=42 chromosomes belong to *P. pseudo-orientale* species. 42 plants were tetraploid with 2n=4x=28 chromosomes (*P. orientale*) and 4 plants were diploid with 2n=2x=14 chromosomes (*P. bracteatum).* The populations used in the study were collected from many different parts of Turkey, so it was not possible that they could pollinate each other. Therefore, no intermediate species with different chromosome numbers were observed.

In this study, karyotype analysis of *Oxytona* section belonging to *Papaveracea* family was made based on the results of karyotype analysis performed by Lavania and Srivastava [17]. The homologous chromosome pairing of the species with arm ratios close to Lavania and Srivastava [17] was applied. Genom of *P. bracteatum* species is diploid and named A (Fig. 2). Similar to, genom of *P. orientale* is tetraploid and named A, B and genom of *P. pseudo-orientale* is hexaploid and named A, B, C (Fig. 3,4). Karyotype analysis results of three species were shown in Table 2, 3 and 4.

Idiogram of each genom was done based on arm lenghts of the chromosomes. The longest arm is placed to the first place (Fig. 5).

There are many studies conducted to date on the species in the *Oxytona* section of the *Papaveracea* family. These studies are not only studies to determine the alkaloid content, but also cytogenetic studies for species identification. Especially two researchers, Cullen [27] and Goldblatt [2], have carried out important cytogenetic studies on plants belonging to the Oxytona section. Cullen [27] and Goldblatt [2] determined important findings in their studies on the *Oxytona* section of wild species in Turkey. While Cullen [27] stated in his study that there are all three species belonging to the *Oxytona* section in our country, Goldblatt [2] did not provide a written document that he encountered *P. bracteatum* in his study conducted in a wide geographical area starting from Central Anatolia and extending to the Hakkari region. In our study, the accessions 23 (Field Crops Department/Ankara University), 99 (Field Crops Department/Ankara University), 104 (Fesleğen Village Göksun Plateau/Niğde) and 149 (Field Crops Department/Ankara University) had $2n=14$ chromosome numbers of *P. bracteatum.* In this case, our works hows similarity to Cullen's work, but contradicts with Goldblatt's work. In addition, in the past written studies it was mentioned that this species (*P. bracteatum*) was known as *P. lasiothrix* [6]. Fedde [5] argued and said that *P. lasiothrix* was originally belong to the group *P. pseudo-orientale*, so the naming was wrong. He stated that there a son for this error was due to the highly similar morphological features between *P. bracteatum* and *P. pseudo-orientale.*

According to the study, *P. pseudo-orientale* (2n = 42) is commonly seen in Fesleğen Village Göksun Plateau/Niğde and can be also seen in Yıldız Mountain/Sivas, Ovacık Mercan Valley/Tunceli locations while *P. orientale* (2n = 28) wasfoundto be wide spread in the Ovacık Mercan Valley/Tunceli

region. It also spreads between the Ovacık Mercan Valley/Tunceli and Fesleğen Village Göksun Plateau/Niğde.

As a result of this study, the chromosome characteristics of the three species were defined based on karyotypes. It is considered that these definitions may be useful in the subsequent floristic, monographic and cytological studies.

TABLE I. Origin of the accessions

Origin of Collection*	Plant Number	Altitude
A	5	2237
B	7	2019
C	23, 99, 149	800
D	33, 34, 35, 36, 39, 40,41, 44, 47,50, 51, 65, 69, 70	2200
E	71, 74, 75, 101, 102, 104,106, 107,112, 113, 114,115, 116, 120, 121, 122, 123, 126, 130, 131, 132, 135, 136,137, 138, 142, 145, 146, 147, 153, 156, 157, 159, 161, 162, 163,165, 166, 169, 170, 181, 183, 184, 186, 190, 191, 192, 196,199, 200, 202, 203, 204, 206, 207, 209, 215, 216, 217, 218, 222, 229, 230, 231, 234, 235, 236, 239, 240, 244	1500

***A** = *Yıldız Mountain/Sivas,* **B** = *Tecer Mountain/Sivas,* **C** = *Ankara University,* **D** = *Ovacık Mercan Valley/Tunceli,* **E** = *Fesleğen Village/Niğde*

TABLE II. Measured lenghts of chromosome arms of *Papaver bracteatum*

Chrom. Number	Long Arm (μm)	Short Arm (μm)	Arm Ratio
1	1.4	1.1	1.27
2	1.7	1.0	1.7
3	1.4	0.9	1.55
4	1.4	0.8	1.75
5	2.1	0.7	3
6	1.4	0.8	1.75
7	2.1	0.6	3.5
8	1.8	1.1	1.6
9	1.7	0.9	1.88
10	1.4	1.2	1.16
11	1.6	0.7	2.28
12	1.4	0.6	2.33
13	1.4	0.9	1.55
14	1.8	1.2	1.5

TABLE III. Measured lenghts of chromosome arms of *Papaver orientale.*

Chrom. Number	Long Arm (μm)	Short Arm (μm)	Arm Ratio
1	0.7	0.4	1.75
2	0.8	0.4	2
3	0.7	0.6	1.16
4	0.8	0.4	2
5	0.7	0.3	2.33
6	0.7	0.4	1.75
7	0.8	0.5	1.6
8	0.8	0.8	1
9	0.4	0.3	1.33
10	0.6	0.2	3
11	0.5	0.4	1.25
12	0.6	0.2	3
13	0.7	0.4	1.75
14	0.5	0.3	1.66
15	0.4	0.3	1.33
16	0.7	0.5	1.25
17	0.6	0.4	1.5
18	0.5	0.4	1.25
19	0.7	0.6	1.16
20	0.8	0.8	1
21	0.7	0.4	1.75
22	0.9	0.4	2.25
23	0.7	0.4	1.75
24	0.7	0.4	1.75
25	0.7	0.3	2.33
26	0.9	0.6	1.5
27	0.7	0.4	1.75
28	0.8	0.4	2

TABLE IV. Measured lengths of chromosome arms of *Papaver pseudo-orientale.*

Chrom. Number	Long Arm (μm)	Short Arm (μm)	Arm Ratio
1	0.5	0.4	1.5
2	0.6	0.4	1.5
3	0.5	0.3	1.66
4	0.4	0.2	2.0
5	0.6	0.4	1.5
6	0.6	0.3	2.0
7	0.5	0.3	1.66
8	0.5	0.2	2.5
9	0.4	0.3	1.33
10	0.5	0.2	2.5
11	0.5	0.5	1.0
12	0.5	0.3	1.66
13	0.5	0.3	1.66
14	0.5	0.3	1.66
15	0.5	0.2	2.5
16	0.6	0.4	1.5
17	0.5	0.4	1.25
18	0.5	0.3	1.6
19	0.5	0.3	1.66
20	0.6	0.3	2.0
21	0.5	0.3	1.66
22	0.3	0.3	1.0
23	0.4	0.4	1.0
24	0.4	0.3	1.33
25	0.6	0.3	2.0
26	0.6	0.4	1.5
27	0.5	0.3	1.66
28	0.6	0.3	2.0
29	0.4	0.3	1.33
30	0.4	0.4	1.0
31	0.5	0.3	1.66
32	0.5	0.2	2.5
33	0.4	0.4	1.0
34	0.5	0.3	1.66
35	0.5	0.3	1.66
36	0.6	0.3	2.0
37	0.4	0.3	1.33
38	0.5	0.4	1.5
39	0.5	0.2	2.5
40	0.5	0.2	2.5
41	0.4	0.4	1.0
42	0.4	0.3	1.33

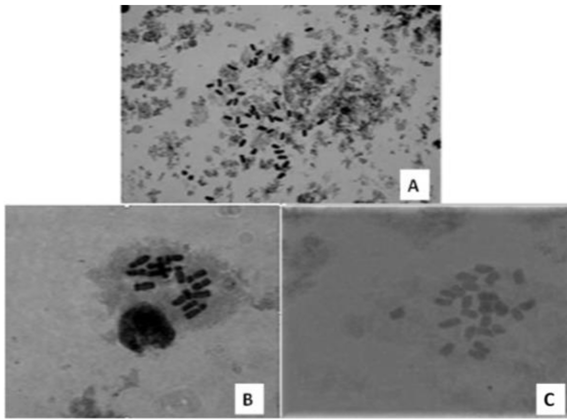

Fig. 1. ***A:*** *Chromosomes of a plant from Fesleğen Village/Niğde region. Chromosome number is 2n=42 (Papaver pseudo-orientale).* ***B:*** *Chromosomes of a plant from Ankara University. Chromosome number is 2n=14 (P. bracteatum nice cell for chromosome count).* ***C:*** *Chromosomes of a plant from Fesleğen Village/Göksun-Niğde region. Chromosome number is 2n=28 (P. orientale)*

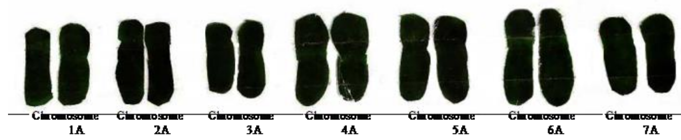

Fig. 2. *Karyotype Analysis of Papaver bracteatum*

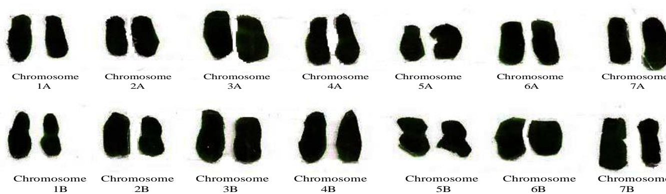

Fig. 3. *Karyotype Analysis of Papaver oriental*

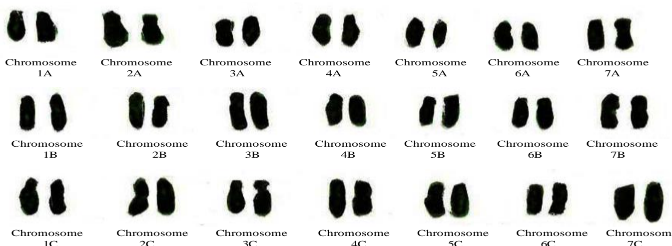

Fig. 4. *Karyotype Analysis of Papaver pseudo-orientale.*

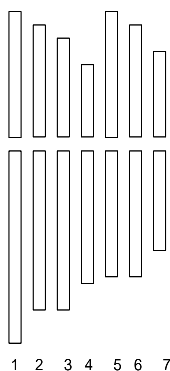

Fig. 5. *Idigram of section Oxytona according to the level of haploid (n) ploidy*

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