



Citation: Aykut Yilmaz, Yudum Yeltekin (2021) Karyomorphology of some *Crocus* L. taxa from Uşak province in Turkey. *Caryologia* 74(4): 129-134. doi: 10.36253/caryologia-1354

Received: June 29, 2021

Accepted: November 30, 2021

Published: March 08, 2022

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Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Competing Interests: The Author(s) declare(s) no conflict of interest.

Karyomorphology of some *Crocus* L. taxa from Uşak province in Turkey

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Abstract. The increasing number of new taxa for each day and the presence of the samples exhibiting variable characters depend on this situation make very problematic the genus *Crocus* as taxonomic and phylogenetic. For this reason, the many studies based on PCR, DNA barcoding and cytogenetics are applied to provide contribution for taxonomic problems and phylogenetic relationships of the genus *Crocus*. In this study, detailed karyotypic investigation of four taxa (*C. pallasii* Goldb. subsp. *pallasii*, *C. olivieri* J.Gay subsp. *olivieri*, *C. fleischeri* J.Gay and *C. uschakensis* Rukšans) belonging to Uşak province in Turkey was carried out and compared with the studies made previously. The somatic chromosome numbers of studied taxa were found to be $2n=14$ for *C. pallasii* subsp. *pallasii*, $2n=8$ for *C. olivieri* subsp. *olivieri*, $2n=20$ for *C. fleischeri* and $2n=20$ for *C. uschakensis*. *C. uschakensis* has only satellite on the short arm of chromosome 7. Some differences with previous studies in aspect of chromosome number and morphology were determined in this study. Furthermore, there is no enough literature information on *Crocus uschakensis* and it was provided with this study based on detailed chromosomal investigation.

Keywords: *Crocus*, cytogenetics, *C. pallasii* subsp. *pallasii*, *C. olivieri* subsp. *olivieri*, *C. fleischeri* and *C. uschakensis*.

INTRODUCTION

The genus *Crocus* L. belonging to the family Iridaceae is represented by about 200 species and show distribution from western Europe and north west Africa to western China (Mathew 1982; Harpke *et al.* 2016; Saxena 2016; Roma-Marzio *et al.* 2018). Especially, the Mediterranean region extending eastward into the Irano-Turanian region is the place containing the majority of the species in the genus *Crocus* (Saxena 2016). Turkey is one of the most important countries with species number and endemism rate for the *Crocus* taxa.

The genus *Crocus* is systematically very problematic. The variable characters caused by environmental factors due to extensive variety of habitats is the one of the most important reasons for taxonomic problems. Furthermore, intermediate characters caused by introgression as a result of hybridization is observed frequently in closely related species (Harrison and Lar-

son 2014; Kerndorff *et al.* 2016; Yılmaz 2021b). The number of the taxa belonging to the genus *Crocus* have recently doubled with the detailed field studies particularly in Turkey (Addam *et al.* 2019). While Uslu *et al.* (2012) states that there are almost 70 *Crocus* taxa which is their 31 endemic to Turkey, Gedik *et al.* (2017) states that Turkey is represented by 132 taxa which is their 108 endemic. However this caused increase the taxonomic problems at the infraspecific level. As a result, it is proposed that subspecies status can not be maintained and anymore must be categorized as species (Harpke *et al.* 2016; Addam *et al.* 2019).

Another important situation which increase taxonomic problems in the genus *Crocus* is the changes observed in the chromosome number. Studies on the karyotypes of *Crocus* taxa show chromosome number changes from $2n=6$ to $2n=70$ within the genus (Brighton *et al.* 1973; Uslu *et al.* 2012; Harpke *et al.* 2013). Furthermore, it was observed that some species from different localities show variation in chromosome number (Uslu *et al.* 2012; Karamplianis *et al.* 2013).

All of these makes problematic the genus and doubtful the species identification within the genus. In addition to cytogenetic studies, many molecular studies based on different PCR methods and DNA barcoding containing nuclear and cpDNA sequences show reality of this situation (Petersen *et al.* 2008; Harpke *et al.* 2013; Erol *et al.* 2014; Yılmaz 2021 a,b).

In this study, the *Crocus* taxa (*C. pallasii* subsp. *pallasii*, *C. olivieri* subsp. *olivieri*, *C. fleischeri* and *C. uschakensis*) from Uşak province were detailed examined based on their somatic chromosome numbers, karyotypic descriptions, length ranges, haploid complements and other morphometric parameters such as I^C (Centromeric index), A_1 (Intrachromosomal asymmetric index), A_2 (Interchromosomal asymmetric index).

One of the most important reasons for the choosing this region in the study is that there is not enough information about *Crocus* taxa in Uşak which is one of the regions with the highest species diversity, in addition to cytological data.

MATERIALS AND METHODS

Plant samples examined in this study were collected from Uşak province in Turkey. There are four taxa containing *C. pallasii* subsp. *pallasii*, *C. olivieri* subsp. *olivieri*, *C. fleischeri* and *C. uschakensis* in this study (Table 1). Root tips for plant samples belonging to each taxa were used to provide somatic metaphase chromosomes. Firstly, root tips that are convenient for

working were put into small glass bottles and then pre-treated in α -monobromobromonaphthalene for 14-16 h at 4°C. After the first treatment, root tips were fixed with Carnoy solution for overnight. Fixed root tips were transferred to bottles with 70% alcohol and stored at 4°C until use. After the all treatments, hydrolysis with 1 N HCl solution was done at 60°C between 14-16 min. Prior to staining, root tips were washed with distilled water. They were stained with 2% aceto-orcein for two hours and then squashed with 45% acetic acid to obtain metaphase chromosomes. Preparations containing the best metaphase chromosomes were photographed using LEICA DM LB2 microscope with camera. The measurements detailed based on small-long arm length and arm ratio were made for each taxa represented by the least five plates. Chromosomes for each taxa examined were classified according to the nomenclature of Levan *et al.* (1964) and Stebbins (1971). In addition to somatic chromosome number, karyotypic description and length ranges, karyotype asymmetry parameters including centromeric index (I^C), intrachromosomal asymmetric index (A_1) and interchromosomal asymmetric index (A_2) were calculated according to Romero Zarco (1986).

RESULTS AND DISCUSSION

All samples examined were provided from Uşak province in Turkey (Table 1). This study aims to analyze the karyotypes of four *Crocus* taxa and to determine the relationships among the taxa studied in addition to *Crocus* taxa examined previously according to chromosome number and other morphometric parameters. At the same time, an important *Crocus* species: *C. uschakensis* which is not well known and not have sufficient literature information was examined for the first time in detail.

The following cytological features belonging to four *Crocus* taxa examined were observed in this study.

Table 1. Species names, localities and chromosome numbers of studied species.

Species	Locations	Somatic chromosome number
<i>C. pallasii</i> subsp. <i>pallasii</i>	5-10 km after Kaşbelen/Uşak	2n=14
<i>C. olivieri</i> subsp. <i>olivieri</i>	Kent forest/Uşak	2n=8
<i>C. fleischeri</i>	5-10 km after Kaşbelen/Uşak	2n=20
<i>C. uschakensis</i>	5-10 km after Kaşbelen/Uşak	2n=20

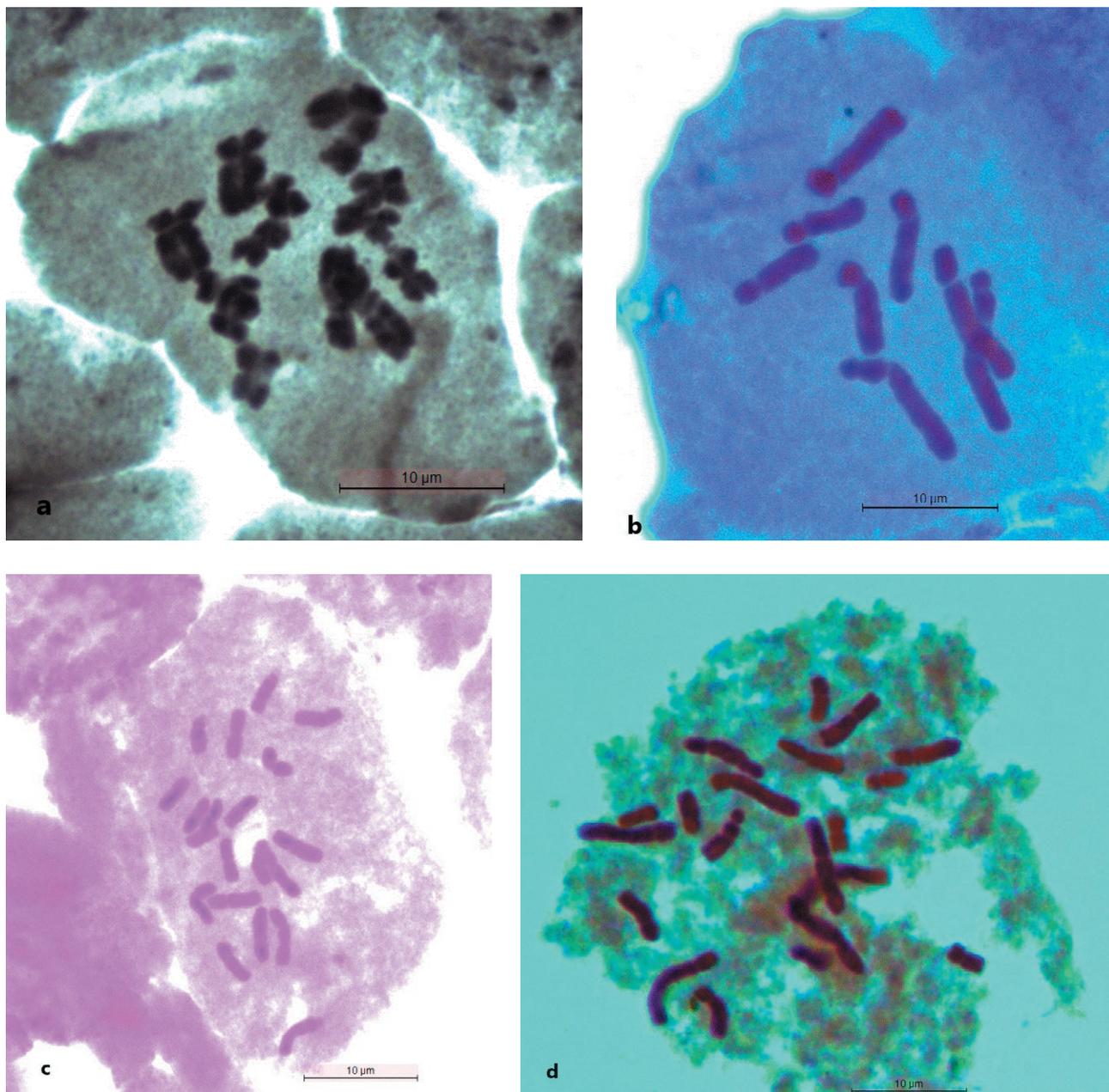


Figure 1. Somatic chromosomes of (a) *C. pallasii* subsp. *pallasii*; (b) *C. olivieri* subsp. *olivieri*; (c) *C. fleischeri*; (d) *C. uschakensis*.

C. pallasii subsp. *pallasii*

Plant samples for *C. pallasii* subsp. *pallasii* were collected from Kaşbelen around in Uşak province. The chromosome number of *C. pallasii* subsp. *pallasii* was determined as $2n=14$ (Table 1, Figure 1-2). Karyotypic description consists of 10 metacentric and 4 submetacentric chromosomes (4sm+10m) (Table 2).

C. pallasii subsp. *pallasii* evaluated within the series *Crocus* show wide distribution from Serbia and Mac-

edonija to Turkey (Karamplianis *et al.* 2013). Chromosome number have been reported in previous studies as $2n=14$ and $2n=16$ for this taxon (Şopova 1972; Brighton *et al.* 1973; Brighton 1977; Randelovic *et al.* 2007; Candan *et al.* 2009). Furthermore, it was determined the both chromosome numbers in the study based on three different populations of *C. pallasii* subsp. *pallasii* by Karamplianis *et al.* (2013). The results provided from the population belonging to Samos Island show similar-

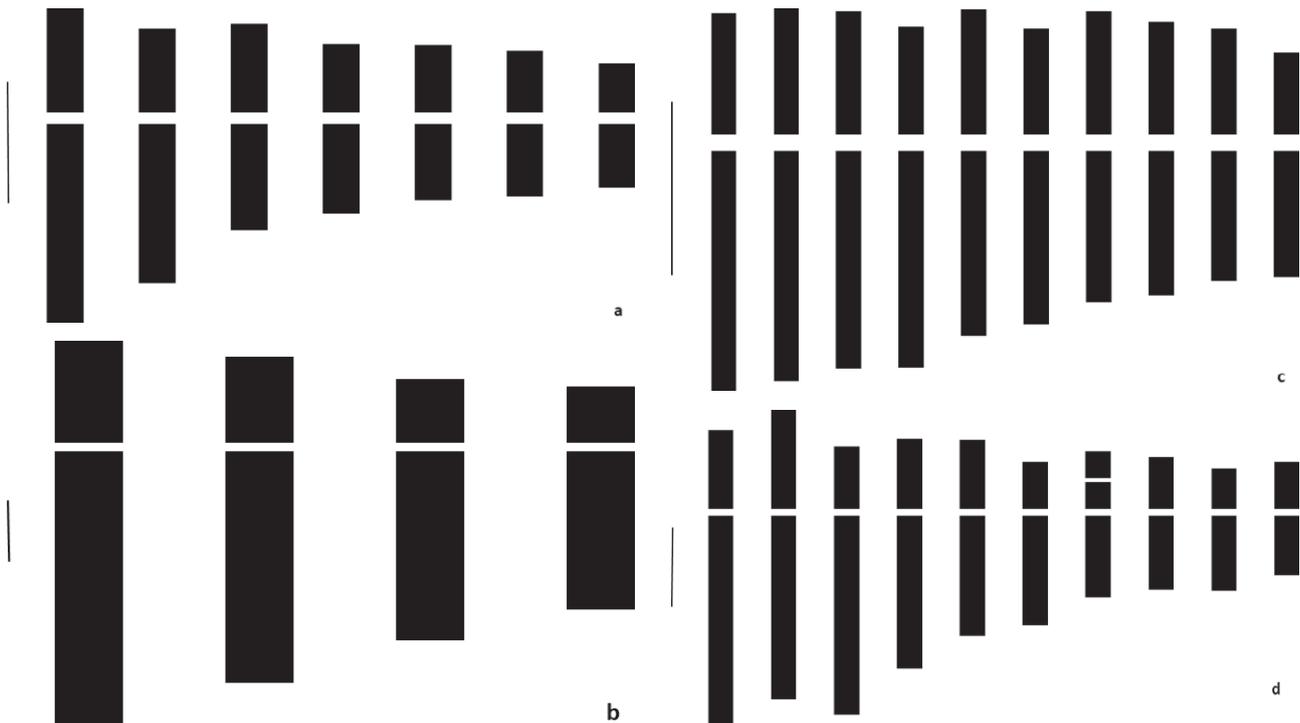


Figure 2. Idiograms of (a) *C. pallasii* subsp. *pallasii*; (b) *C. olivieri* subsp. *olivieri*; (c) *C. fleischeri*; (d) *C. uschakensis*. (Bar : 2 μ m).

ity with this study according to chromosome number ($2n=14$) and karyotypic description (4sm+10m).

C. pallasii subsp. *pallasii* had the smallest chromosomes set (1.93–5.17 μ m) in addition to lowest haploid complement value (22.01 μ m) among the studied taxa (Table 2).

A_1 had the lowest value (0.27) among all the studied taxa, while A_2 had the second highest value (0.36) after *C. uschakensis*. The highest centromeric index value with 41.97 was observed in *C. pallasii* subsp. *pallasii* (Table 2).

C. olivieri subsp. *olivieri*

Crocus olivieri subsp. *olivieri* is distributed in Turkey, Macedonia, Southeast Romania, South Bulgaria, Albania and Greece (Yüzbaşıoğlu 2012).

C. olivieri subsp. *olivieri* chromosome number was found to be $2n=8$ (Table 1, Figure 1-2). All chromosomes belonging to this taxon examined were submetacentric. Chromosome counts for *C. olivieri* subsp. *olivieri* from different locations were done previously and reported as $2n=6$ (Mather 1932; Brighton *et al.* 1973; Uslu *et al.* 2012). In this aspect, chromosome number of this taxon from Uşak location show differences from other study results.

Chromosome length range was between 7.30–12.80 μ m (Table 2). In other words, the longest chromosomes

set was observed in *C. olivieri* subsp. *olivieri*, although it has the least chromosome number in comparison to other taxa examined. Furthermore the second highest haploid complement value with 39.51 μ m was found in this taxon (Table 2). The lowest centromeric index value with 26.43 and highest A_1 value (0.65) were determined in *C. olivieri* subsp. *olivieri* (Table 2).

C. fleischeri

Chromosome number of *C. fleischeri* was found to be $2n=20$ (Table 1, Figure 1-2). *Crocus fleischeri* is distributed South and West Anatolia regions of Turkey. Chromosome number of this taxon which is endemic was previously reported as $2n=20$ (Mathew 1984; Candan *et al.* 2009). Furthermore, it is stated by Candan *et al.* (2009) that all of the chromosomes are submetacentric except 3 chromosomes being metacentric. In this study, karyotypic description of this taxon consist of 8 metacentric and 12 submetacentric chromosomes (12sm+8m) (Table 2).

Chromosome length range and haploid complement value for this taxon have the lowest value after *C. pallasii* subsp. *pallasii* with 2.42–4.22 μ m and 34.35 μ m respectively (Table 2).

Chromosomal asymmetry index, A_1 and A_2 were determined as 0.36 and 0.17, respectively. The lowest A_2

Table 2. Karyotypic descriptions, length ranges and other morphometric parameters of studied *Crocus* species.

Species	Karyotypic description	Length range (μm)	Haploid complement (μm)	I ^C	A ₁	A ₂
<i>C. pallasii</i> subsp. <i>pallasii</i>	4sm+10m	(1.93 – 5.17)	22.01	41.97	0.27	0.36
<i>C. olivieri</i> subsp. <i>olivieri</i>	8sm	(7.30 – 12.80)	39.51	26.43	0.65	0.24
<i>C. fleischeri</i>	12sm+8m	(2.42 – 4.22)	34.35	38.80	0.36	0.17
<i>C. uschakensis</i>	2a+12sm+6m	(2.87 – 7.82)	50.93	34.49	0.47	0.37

value was observed in *C. fleischeri* with 0.17 (Table 2).

C. uschakensis

C. uschakensis is an endemic species and there is not enough information about this taxon. This work represents the first detailed chromosomal study on *C. uschakensis*. Rukšans (2014) states that they observed this taxon on low mountains belonging to North of Uşak. Similarly, we observed and collected this taxon on North parts of Uşak province.

Chromosome number of *C. uschakensis* was found to be $2n=20$ (Table 1, Figure 1-2). which consist of 6 metacentric, 12 submetacentric and 2 acrocentric chromosomes (2a+12sm+6m) (Table 2). Furthermore, satellite was observed on the short arm of chromosome 7. Chromosome length range was between 2.87–7.82 μm (Table 2). In other words, the longest chromosomes set was observed in *C. uschakensis* after *C. olivieri* subsp. *olivieri* which has the least chromosome number among taxa examined. The longest haploid complement was determined in *C. uschakensis* with 50.93 μm . The second lowest centromeric index value with 34.49 and highest A₂ value with 0.37 was observed in this taxon (Table 2).

The variation in chromosome counts for two species were observed in this study. While the chromosome number for *C. pallasii* subsp. *pallasii* have been reported as $2n=14$ and $2n=16$ in previous studies, it was determined $2n=14$ in present study. Similarly, another different chromosome count was found in *C. olivieri* subsp. *olivieri* as previously reported as $2n=6$, whereas in the present study it was found as $2n=8$.

It is observed wide range of variation on the chromosomes counts (from $2n=6$ to 70) and morphology of the species belonging to the genus *Crocus* (Brighton *et al.* 1973; Uslu *et al.* 2012; Harpke *et al.* 2013). The most probably reasons of variations in the chromosome number and morphology of the species are geographical differences, environmental factors caused by locations of taxa, hybridization, polyploidization and aneuploidy. Geographical differences and variations in environmen-

tal factors caused by geographical differences could be reason of chromosome count differences in *C. olivieri* subsp. *olivieri*. Similarly, Karamplianis *et al.* (2013) examining the *C. pallasii* subsp. *pallasii* in three different populations states that chromosome numbers for this taxon change as $2n=14$ and $2n=16$.

Other an important taxon, *C. uschakensis* were examined in detailed according to chromosome number and other morphometric parameters. Furthermore, in addition to contribution for literature based on its karyotype informations determined, it was firstly evaluated the relationships of *C. uschakensis* with other *Crocus* taxa. Besides the first detailed karyotype analysis, satellite chromosome was determined in *C. uschakensis*.

Turkey with 132 taxa which is their 108 endemic is found in very rich region according to species number and diversity in the world (Gedik *et al.* 2017) and accepted as the center of species diversity for the genus *Crocus* (Erol *et al.* 2012; Candan and Özhatay 2013). The genus *Crocus* in the world in comparison to Turkey according to their species number and diversity, it can be said that Turkey is the center of genetic variation for the genus. Furthermore, the high endemism rate for the *Crocus* species make very important the Turkey in aspect of studies on the genus.

In this study, four species belonging to Uşak province located in the Western Anatolia region which is the richest region of Turkey according to species diversity were examined caryologically. One of the most important gains of this study is to obtain literature information on *Crocus uschakensis* which is an endemic species, in addition to determining the species diversity of the region.

ACKNOWLEDGMENTS

The authors would like to thank Uşak University Directorate of Scientific Research Projects (BAP-2021/MF003) for providing financial support and also special thanks to Ahmet Kahraman for helping to identify the plant material.

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