OKU Fen Bilimleri Enstitüsü Dergisi
6(Ek Sayı): 199-210, 2023

OKU Journal of The Institute of Science and Technology, 6(Suppl): 199-210, 2023

Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi

Osmaniye Korkut Ata University Journal of The Institute of Science and Technology

Cytogenetic Investigation of Some *Ranunculus* L. Species Distributed in Bitlis and its Surroundings

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Research Article

Article History:
Received: 26.07.2022
Accepted: 24.06.2023
Published online: 20.12.2023

Keywords:
Ranunculus
Chromosome
Turkey

ABSTRACT

Ranunculus L. has 87 species in Turkey, when subspecies and varieties are included in this number, it is represented by a total of 105 taxa, 24 of which are endemic, the endemism rate is 25.20%. Since many members of the genus *Ranunculus* cannot be distinguished from each other with definite differences, they have been tried to be defined and expressed as species complexes taxonomically. Most of the systematic studies are in the direction of regulating such complexes. It is known that karyological characters are of great importance in the phylogenetic classification of the Ranunculaceae family. In this research, it is aimed to reveal the karyological characteristics of three species which are very similar to each other, in group A of *Ranunculus* subgenus of *Ranunculus* L. in Volume 1 of Flora of Turkey morphologically by using cytogenetic methods. In this context, the karyological characteristics of *R. aquatilis* L., *R. poluninii* P.H.Davis and *R. crateris* P.H.Davis species have been determined. *R. poluninii* and *R. crateris* species are endemic to our country and are Iran-Turanian elements. On the other hand *R. aquatilis* species, is distributed in the East of Turkey. It has been determined that all three species whose chromosomal characteristics are examined are diploid, and their chromosome numbers are x=8, 2n=16. It has also been determined that these three species, which are morphologically similar to each other, are the same in terms of chromosome number but different in terms of chromosome morphology and each species has its own characteristics. With this study, the chromosomal characteristics of these three species have been determined for the first time.

Bitlis ve Çevresinde Yayılış Gösteren Bazı *Ranunculus* L. Türlerinin Sitogenetik Yönden İncelenmesi

Araştırma Makalesi

Makale Tarihçesi:
Geliş tarihi: 26.07.2022
Kabul tarihi:24.06.2023
Online Yayınlanma: 20.12.2023

Anahtar Kelimeler:

ÖZ

Ranunculus L. cinsi Türkiye'de 87 türe sahiptir, bu tür sayısına alttür ve varyeteler de dahil edildiğinde toplamda 105 takson ile temsil edilmekte olup bunların 24'si endemiktir, endemizm oranı % 25,20' dir. *Ranunculus* cinsinin pek çok üyesi kesin farklarla birbirlerinden ayırt edilemediğinden taksonomik olarak tür kompleksleri şeklinde ifade edilip tanımlanmasına

Ranunculus
Kromozom
Türkiye

çalışılmıştır. Sistematik çalışmaların çoğu da bu tür komplekslerini düzenleme yönündedir. Ranunculaceae familyasının filogenetik sınıflandırmasında karyolojik karakterlerin büyük önem taşıdığı bilinmektedir. Bu çalışmada, Türkiye Florası 1. Cildinde bulunan *Ranunculus* L. cinsinin *Ranunculus* altcinsine ait Grup A'da bulunan ve birbirlerine morfolojik olarak çok benzeyen üç türün sitogenetik metodlar kullanılarak karyolojik özelliklerinin ortaya çıkarılması hedeflenmiştir. Bu kapsamda Bitlis ili çevresinden toplanan *R. aquatilis* L., *R. poluninii* P.H.Davis ve *R. crateris* P.H.Davis türlerinin karyolojik özellikleri tespit edilmiştir. *R. poluninii* ve *R. crateris* türleri Ülkemize endemik ve İran – Turan elementidir. *R. aquatilis* türü ise Türkiye'nin Doğusunda yayılış gösterir. Kromozomal özellikleri incelenen üç türün de diploid olduğu ve kromozom sayılarının x=8, 2n=16 olduğu tespit edilmiştir. Morfolojik olarak birbirine benzer olan bu üç türün, kromozom sayısı bakımından aynı fakat kromozom morfolojileri açısından farklı ve her türün kendine özgü özelliklere sahip oldukları belirlenmiştir. Bu çalışma ile birlikte sözkonusu üç türün kromozom özellikleri ilk defa tespit edilmiştir.

To Cite: Almazov S., Sancar PY., Kursat M., Gedik O., Civelek S. Cytogenetic Investigation of Some *Ranunculus* L. Species Distributed in Bitlis and its Surroundings. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi 2023; 6(Ek Sayı): 199-210.

Introduction

The Ranunculaceae has 59 genus and about 2500 species in the world. Members of the family are herbaceous, rarely climbing woody plants. *Ranunculus* L. (Buttercup) is the largest genus of the Ranunculaceae and contains approximately 600 species. Its distribution is nearly worldwide, the greatest number of species occurs in the temperate zones of North and South America, the Alpine regions of Europe, Asia, Australia, New Zealand and New Guinea. The number of species is low in tropical regions, but taxa in these regions are distributed in the high parts of the mountains (Tamura, 1993; Tamura, 1995; Lehnebach, 2008; Özdağ, 2020). In our country, the Ranunculaceae family has 19 genera and 203 species. When subspecies and varieties are added to this number of species, it is represented by a total of 234 taxa. Of these, 62 taxa are endemic and the endemism ratio is 26.5%. *Ranunculus* genus has 87 species in Turkey, when subspecies and varieties are included in this number of species, it is represented by a total of 105 taxa, 24 of which are endemic, the endemism ratio is 25.20 % (Güner Doğan, 2012; Yıldırım and Gül, 2018; Karaman Erkul et al., 2021; Sinan et al., 2021). The genus *Ranunculus* is a group of plants with a high ploidy ratio, composed mostly of polymorphic ones that show a high variation in both morphological and karyological characteristics. In general terms, 40% of this genus is hexaploid, 31% is tetraploid, and 28% is diploid (Goepfert, 1974). As many members of the genus *Ranunculus* in the world, as in Turkey, cannot be distinguished from each other with definite differences, it has been tried to be expressed and defined as species complexes taxonomically. Most of the systematic studies are in the direction of regulating such complexes.

It is known that karyological characters are of great importance in the phylogenetic relationships of the Ranunculaceae family (Tamura, 1995). In this paper, the karyological characteristics of three species in Group A of *Ranunculus* subgenus of the genus *Ranunculus* found in Volume 1 of Flora of Turkey (Davis, 1965) and very similar to each other in morphology were tried to be determined by using cytogenetic methods. Two of these species (*Ranunculus poluninii* P.H.Davis and *Ranunculus crateris*

P.H.Davis) whose karyological characteristics were tried to be determined are endemic to our country, and one (*Ranunculus aquatilis* L.) is a species that is morphologically very similar to these endemic species and is found only around Bitlis province and has a narrow distribution in our country (Davis, 1965; Güner Doğan, 2012).

In this study, it is aimed to bring the karyological data to be obtained by us about the populations of these endemic and narrowly distributed rare species in our country for the first time into the literature.

In addition to aimed, enable the use of karyological data together with morphological and molecular data in solving the systematic problems of the species and thus to be able to recognize the species more systematically with the karyological scientific data to be obtained.

Materials and Methods

Material

The research materials were collected separately as flowering and seed plants during the field studies carried out in Bitlis and its surroundings between May and September of 2021, and the flowering specimens were turned into herbarium material. The seeds collected from the plants to be used in our cytogenetic studies were dried and stored in cloth bags in a moisture-free environment. When collecting seed samples, as many different populations as possible were used for each taxon. Thus, it was analyzed whether there is inter-population chromosomal variation. Detailed locality information of the samples and general appearance are given in Table 1 and Figure 1.

Table 1. Detailed location information in studied taxa

Taxa	Collection date and collector	Detailed localities of populations
R. poluninii	08.09.2021- M.K.	Bitlis, North hill side of Kambos Mountain, rocky places, 1650-1800 m.
R. crateris	16.05.2021-M.K.	Bitlis, road to Nemrut Crater lake, hills, below *Juniperus* populations, 2370-2400 m.
R. aquatilis	26.07.2021- M.K.	Van, Mount Artos, Northern slopes, 2200 m.

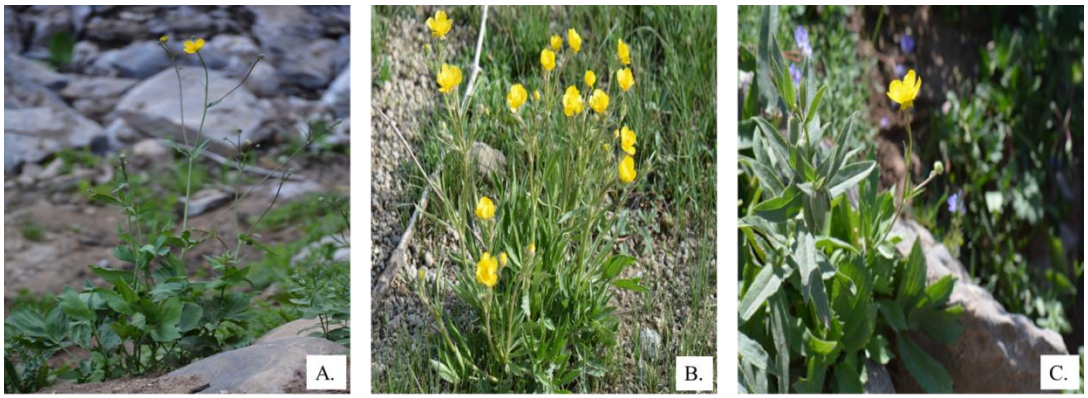

Figure 1. General appearance of the species (**A**-*R. poluninii*, **B**-*R. crateris*, **C**-*R. aquatilis*)

Chromosome Analysis

Within the scope of our cytogenetic studies, the seeds were first left to germinate on moist blotting papers laid in petri dishes in an oven at 28°C. Then, the roots reaching 1–2 cm from the germinated seeds were cut and kept in 5% colchicine for 3 hours at room temperature and subjected to pretreatment (Elçi, 1982; Yilmaz Sancar et al., 2021). At the end of the period, the root tips were taken into a carnoy fixative (3:1) and kept in the refrigerator at +4 ºC for 24 hours and then hydrolyzed in 1N HCl at room temperature for 3-5 minutes. Root tips removed from hydrolysis were stained with Feulgen dye for 1 hour in a dark environment at room temperature and washed 2-3 times with tap water. For the preparation, the growth meristem part was fragmented with a sharp razor blade in a drop of 45% acetic acid-orcein mixture dropped on the slide, the coverslip was closed and examined under the microscope (Yilmaz Sancar and Civelek, 2012). Photographs of the best three somatic cells for each species were taken under a Zeiss brand Primostar model microscope with a 100's lens. Chromosome lengths of 3 photographs with the most prominent chromosome distribution during metaphase were measured. Tables and ideograms were created by calculating the average. The long and short arms of the chromosomes were measured with the special program of the microscope, and the obtained data were recorded in tabular form. Terminology of the Levan was used to locate the centromere (Levan et al., 1964).

Results

This research, values such as ploidy levels, karyotype formula, chromosome length range, and total karyotype length were determined and given in the tables in detail (Table 2-5). Also, the metaphase chromosomes and haploid ideograms of these three *Ranunculus* taxa are presented Figures 2–4.

Table 2. Somatic chromosome number (2n), ploidy level, karyotype formula, ranges of chromosome length, total karyotype length (TKL) for the studied taxa.

Taxon	2n	Ploidy level	Karyotype formula	Chromosome length range (µm)	TKL(µm
R. aquatilis	16	2x	1m+6sm+1st	14.11-9.12	83.75
R. poluninii	16	2x	2m+4sm+2st	16.92-9.24	94.45
R. crateris	16	2x	2m+2sm+4st	15.03-8.38	86.36

(m: median M: noktalı median Sm: submedian St: subterminal)

R. aquatilis

The chromosome number of *R. aquatilis* species was determined as 2n=2x=16 (x=8) and the chromosome formula was determined as 1m+6sm+1st. It was observed that the chromosome length of the population varied between 9.12-14.11 microns, and there were satellites in chromosomes I and III. From karyotypes obtained from well-distributed metaphases; somatic chromosome number, ploidy level, karyotype formula, chromosome length range and total chromosome length (TCL) are determined and showed in Table 2; chromosome length, long arm, short arm, arm ratio, centromere

index, relative height and centromere status are determined and showed in Table 3. In addition, the metaphase images of the chromosomes and the haploid ideograms are shown in Figure 2.

Table 3. Karyomorphological parameters (relative length, arm ratio and centromeric index) of *R. aquatilis* (m: median M: noktalı median Sm: submedian St: subterminal).

Pair No	Total length C (µm)	Long arm L (µm)	Short arm S (µm)	Arm ratio L/S	Centromeric index İ	Type
1	14.11	7.33	6.78	1.08	0.48	m
2	10.92	7.64	3.28	2.32	0.30	sm
3	10.66	7.38	3.28	2.25	0.30	sm
4	10.12	6.54	3.58	1.82	0.35	sm
5	10.01	7.55	2.46	3.06	0.24	st
6	9.50	6.32	3.18	1.98	0.33	sm
7	9.31	6.51	2.80	2.32	0.30	sm
8	9.12	5.98	3.14	1.90	0.34	sm

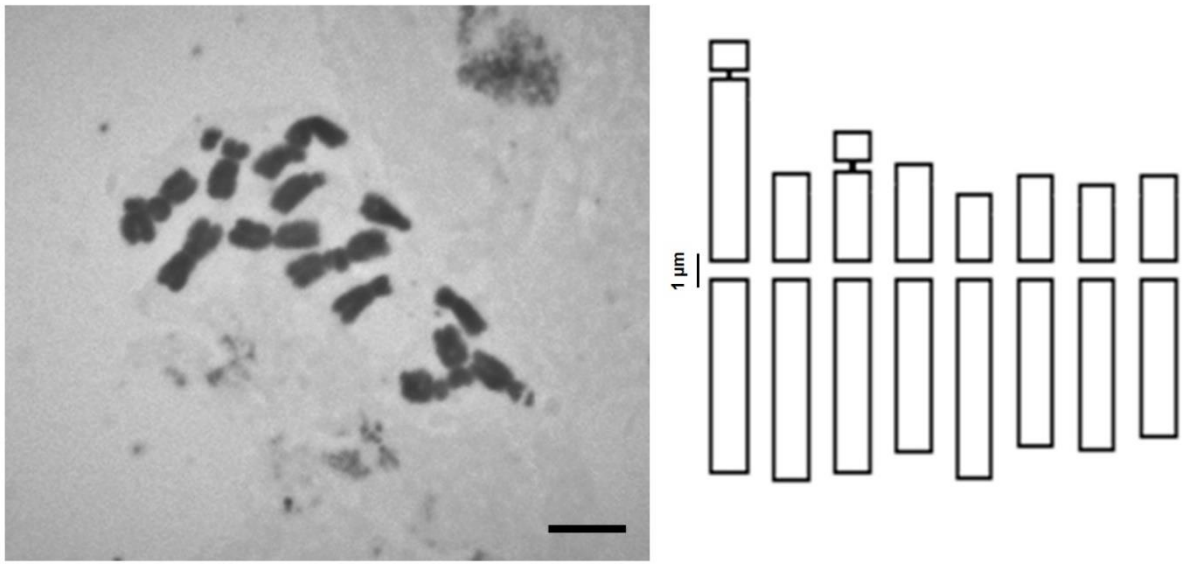

Figure 2. Metaphase chromosomes and haploid ideograms of *R. aquatilis* (Scale:10 µm).

R. poluninii

The chromosome number of *R. poluninii* species was determined as 2n=2x=16 (x=8) and the chromosome formula was determined as 2m+4sm+2st. It was observed that the chromosome length of the population varies between 9.24 -16.92 microns, and there is a satellite in chromosome VIII. From karyotypes obtained from well-distributed metaphases; somatic chromosome number, polyploid level, karyotype formula, chromosome length range and total chromosome length (TCL) are determined and showed in Table 2; chromosome length, long arm, short arm, arm ratio, centromere index, relative height and centromere status are determined and showed in Table 4. In addition, the metaphase images of the chromosomes and the haploid ideograms are shown in Figure 3.

Table 4. Karyomorphological parameters (relative length, arm ratio and centromeric index) of *R. poluninii* (m: median M: noktalı median Sm: submedian St: subterminal).

Pair No	Total length C (µm)	Long arm L (µm)	Short arm (µm) S	Arm ratio L/S	Centromeric index İ	Type
1	16.92	8.83	8.09	1.10	0.47	sm
2	13.87	8.53	5.34	1.59	0.38	m
3	12.19	8.96	3.23	2.77	0.26	sm
4	11.88	7.44	4.44	1.67	0.18	m
5	10.86	7.77	3.09	2.51	0.28	sm
6	10.19	7.14	3.05	2.34	0.29	sm
7	9.30	7.28	2.02	3.60	0.21	st
8	9.24	7.08	2.16	3.27	0.23	st

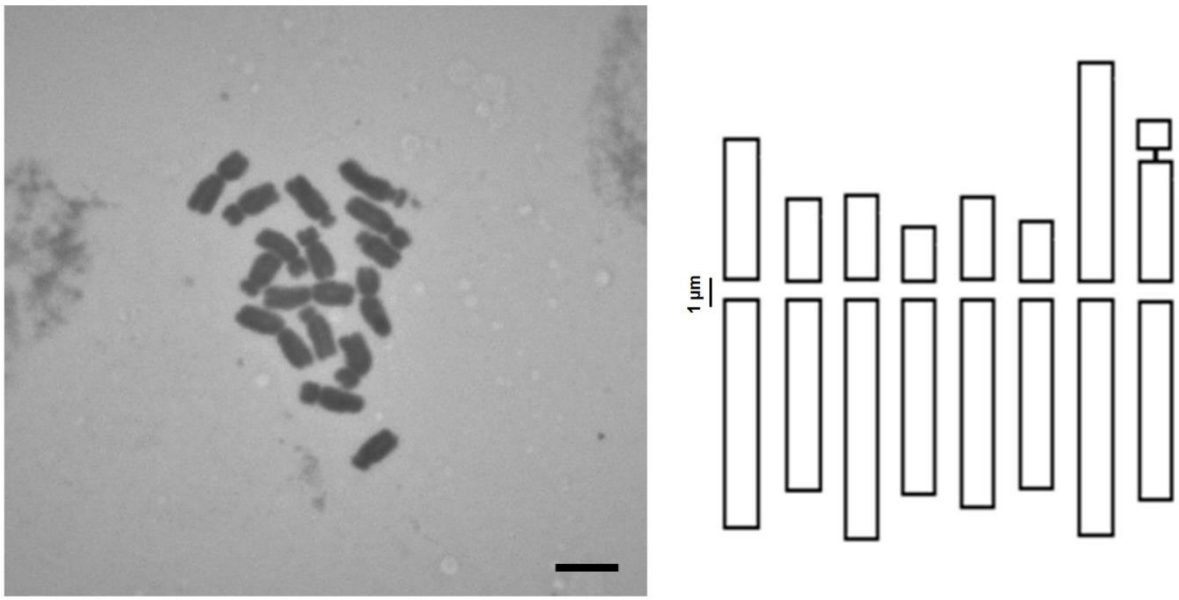

Figure 3. Metaphase chromosomes and haploid ideograms of *R. poluninii* (Scale:10 µm).

R. crateris

The chromosome number of *R. crateris* was determined as 2n=2x=16 (x=8) and the karyotype formula was determined as 2m+2sm+4st. It was observed that the chromosome length of the population varies between 8.38-15.03 microns, and there is a satellite in chromosome VI. From karyotypes obtained from well-distributed metaphases; somatic chromosome number, polyploid level, karyotype formula, chromosome length range and total chromosome length (TCL) are determined and showed in Table 2; chromosome length, long arm, short arm, arm ratio, centromere index, relative height and centromere status are determined and showed in Table 5. In addition, the metaphase images of the chromosomes and the haploid ideograms are shown in Figure 4.

Table 5. Karyomorphological parameters (relative length, arm ratio and centromeric index) of *R. crateris* (m: median M: noktalı median Sm: submedian St: subterminal).

Pair No	Total length C (µm)	Long arm L (µm)	Short arm S (µm)	Arm ratio L/S	Centromeric index İ	Type
1	15.03	7.99	7.04	1.13	0.46	m
2	12.43	7.75	4.68	1.65	0.37	m
3	11.05	8.61	2.44	3.52	0.22	st
4	10.52	7.50	3.02	2.48	0.28	sm
5	10.42	7.19	3.23	2.27	0.30	sm
6	9.54	7.49	2.05	3.65	0.21	st
7	8.99	7.08	1.91	3.70	0.21	st
8	8.38	6.57	1.81	3.62	0.21	st

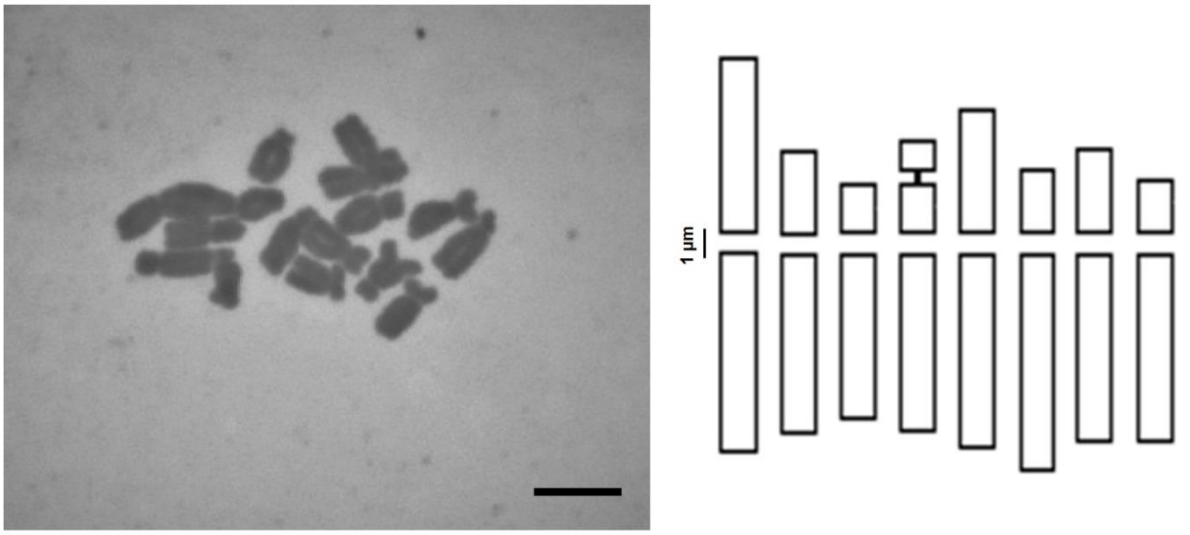

Figure 4. Metaphase chromosomes and haploid ideograms of *R. crateris* (Scale:10 µm).

Discussions

The genus *Ranunculus* are polymorphic plants with a high polyploidy rate, with high variation in both morphological and karyological characteristics. In general terms, 40% of this genus is hexaploid, 31% is tetraploid, and 28% is diploid (Goepfert, 1974). Since many members of the genus *Ranunculus* in the world as well as in our country cannot be distinguished from each other with definite differences, it has been tried to be expressed and defined as species complexes taxonomically. Most of the systematic studies are in the direction of regulating such complexes. For example, a series of studies have been conducted for the *Ranunculus auricomus* L. complex (Julin, 1977; Julin, 1978). *Ranunculus dissectus* M.Bieb., one of the rhizome *Ranunculus* in Turkey, is such a complex species (Davis, 1960; Tan, 1984; Baytop and Özhatay, 1975).

Modern biosystematic research is mainly based on molecular studies, but morphological and cytogenetic studies continue to provide basic data for each organism. Many cytogenetic studies have

been conducted in the last decade and these studies have provided basic data for plant systematics and evolutionary analyses (Stace, 2000; Baltisberger and Widmer, 2009). In spite of their taxonomic importance, numbers of chromosomes and features are known for only about 25% of all angiosperms (Bennett, 1998). Also, the accuracy of many reported chromosome numbers is questionable because they depend on weak or incorrect data (such as inaccurate counting, calculation, observation). This situation decreases the usefulness of the results, especially in taxonomic problematic groups where numbers of chromosomes are usually variable.

In addition to variation in chromosome number, cytogenetic data may show variation in chromosome morphology also (Sharma and Sen, 2002). The most important morphological character of a chromosome is the position of the centromere on the chromosome (Levan et al., 1964). The definition of chromosome morphology has proven to be a strong method for characterizing genomes in plants as well as animals, including humans. Cytogenetic data supply background information for several fields, as demonstrated by the "taxonomic significance of karyology at the genus and subgenus level" in Geraniaceae (Albers and Walt, 2007). Karyological data can also assist in the interpretation of results from molecular studies. (Johansson, 1998; Schuettpelz et al., 2002; Crawford et al., 2005; Hörandl et al., 2005). Cytogenetic data, together with morphological characters, helped to solve taxonomic problems in tracing the origin of hybrids and even provided clarity of possible taxonomic decisions (Bailey and Stace, 1992).

Stace (2000) suggested that three basic conditions must be met for cytogenetic studies to be convenient: Plants examined should be collected from natural areas, some of the studied samples should be kept in herbariums, and chromosome counts should be based on a lot of plants and cells. In addition, different populations close to the location of the studied taxon and other populations collected from different geographical regions should be included in the study (Stace, 2000).

The Ranunculaceae family is a middle-sized plant family with many primitive characters, but at the same time presenting special and advanced features. Cytogenetic features are thought to be of high importance in the evolutionary relationships of Ranunculaceae (Tamura, 1995). The basic chromosome number in *Ranunculus* is usually x=7 or x=8, but the most common is x=8, and this number is considered the basic chromosome number of the genus (Goepfert, 1974). We counted 16 chromosomes in each species we examined that are all diploid and basic chromosome number is 8. Polyploidy is common and differences in ploidy levels can occur even within species (Küpfer, 1974; Baltisberger, 1981; Huber, 1988; Baack, 2005; Hörandl et al., 2005). Karyotypes vary considerably within the genus and even non-closely related species can interbreed, at least under experimental conditions (Goepfert, 1974; Vuille and Küpfer, 1985; Baack, 2004; Baltisberger, 2005). For this reason, hybridization and polyploidy may play an important role in the speciation and evolutionary process of *Ranunculus*. (Rossello and Castro, 2008; Goepfert, 1975).

In this paper, the karyological characteristics of three species in Group A of the *Ranunculus* subgenus of the genus *Ranunculus* L. found in the 1st Volume of Flora of Turkey and very similar to each other

in morphology were tried to be determined by using cytogenetic methods (Davis, 1965). Two of these three species (*Ranunculus poluninii, Ranunculus crateris*) whose karyological features are tried to be determined are endemic to our country, distributed in Bitlis, Muş, Hakkari and Van provinces, and one of them (*Ranunculus aquatilis*) is morphologically very similar to these two endemic species and only found around the province of Bitlis and has a narrow distribution in our country.

The photographs and ideograms of the appearance of the chromosomes in the metaphase plate of the three species (*R. aquatilis, R. poluninii* and *R. crateris*) studied within the scope of our research, and the total length, relative length, arm index and centromere status of the chromosomes are given in detail in the results section. All three species whose chromosomal characteristics were examined were diploid, and their chromosome numbers were x=8, 2n=16. This is consistent with all previous records in the literature (Goepfert, 1974; Goepfert, 1975; Küpfer, 1974; Vuille and Küpfer, 1985; Tamura, 1993; Tamura 1995; Stace, 2000; Baack, 2004; Baltisberger and Widmer, 2009; Rossello and Castro, 2008). The basic chromosome number in *Ranunculus* is usually x=7 or x=8, with the latter much more frequent and the genus is 40% hexaploid, 31% tetraploid, and 28% diploid (Goepfert, 1974).

Polyploidy and aneuploidy chromosome variations were not found in the studied populations of *R. aquatilis, R. poluninii* and *R. crateris* species. It was found that these three species, which are morphologically similar, are different in terms of chromosome morphology and each species has its own characteristics.

In this research, the chromosomal (karyological) characteristics of three narrowly distributed plants of the Ranunculaceae family were determined for the first time. It is known that karyological characters are of big significance in determining their evolutionary relationships and, systematics species boundaries of Ranunculaceae. Our karyological data will help to explicaton the results obtained from systematic and genetic studies on Ranunculaceae members and to make possible taxonomic decisions. (Johansson, 1998; Schuettpelz et al., 2002; Crawford et al., 2005; Hörandl et al., 2005).

Acknowledgment

This work was supported by the Firat University Scientific Research Projects Coordination Unit [grant number: FF.20.09].

Conflict of Interest

The authors declare no conflict of interest.

Author's Contribution

SA and SC designed the study. MK collected plant samples from in the field. PYS and OG performed the methodology. The original draft was written by PYS and SC. All authors read and agreed the final version of manuscript.

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