MORPHOLOGICAL AND KARYOLOGICAL STUDY OF THE SPECIES OF THE FAMILY GERANIACEAE JUSS. OF ARMENIAN FLORA

R. G. ADAMYAN 1*, A. G. GHUKASYAN 2**, A. V. POGHOSYAN 1***

1 Chair of Botany and Mycology, YSU, Armenia
2 Institute of Botany after A. Takhtajyan NAS RA, Armenia

In connection with taxonomic study of representatives of the Geraniaceae family of Armenian flora. Usually during the species identification the structure of the root system, life form, leaf morphology, flower structure, inflorescence, carpels structure are taken into account. The karyological research of the genus Geranium growing in Armenian flora has revealed the following: for 8 species we discovered diploid and tetraploidy cytoraces. The basic chromosome numbers \(x = 9, 10, 13, 14\) shows up in the genus Geranium.

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Introduction. The Geraniaceae Juss. family is rather complicated from taxonomic point of view and includes about 10 genera and 700 species [1]. Majority of species are growing in temperate zone of the Earth and in high mountains of tropical zone. The species are characterized by polymorphism, which makes it difficult to identify them. The Geraniaceae family is represented by two genera and 26 species (Geranium – 20 species, Erodium – 6) in Armenia. These species are distributed in 8 and 2 sections in Geranium and Erodium genera correspondingly. One additional species from each genera can be found in the territory of the republic, as they grow in adjacent territories, and their ecological features allow them to find favorable conditions in Armenia. In connection with the taxonomic processing of representatives of the family Geraniaceae in Armenia an analysis of the diagnostic features species of the family was carried out. The main features that play a role in the construction of dichotomous keys for determining species and sections are identified. Their taxonomic significance has been assessed [2].

Determination of chromosome numbers is the first step in any karyological study. As a result of the accumulation of factual material, this level opens up wide possibilities for using karyological data in systematics. The study of chromosome numbers, in some cases, makes it possible to clarify the issues of speciation and

* E-mail: radamyan@ysu.am
** E-mail: anyaghukasyan@gmail.com
***E-mail: astchik@ysu.am
phylogenetic relationships. The accumulation of data on chromosome numbers makes it possible to carry out a more differentiated approach to understanding a taxon, species, or even family.

Information about the chromosome numbers of species, growing on the territory of Armenia, is presented in the atlas “Chromosomal numbers of flowering plants of the Armenian flora” [3], which summarizes data on 900 species from more than 2000 populations, 51 families belonging to 320 genera. Until now, representatives of the family Geraniaceae of the Armenian flora have not been studied karyologically.

Materials and Methods. The main method for study of the taxonomy of the family is the comparative morphological one, supplemented by karyological and botanical-geographical methods. The morphological characteristics of the species were studied on herbarium specimens and on fresh material. The herbarium collections kept at the Institute of Botany after A. Takhtajyan National Academy of Sciences of the Republic of Armenia (ERE), the Botanical Institute of the Russian Academy of Sciences (LE), the Museum of Natural History of Vienna (W) and the Chair of Botany and Mycology of YSU (ERCB) were studied, as well as own and previously unprocessed collections of species of the family Geraniaceae. Regional “Flora” and databases of Plants for a future were studied.

Database search.: http://www.ibiblio.org/, the Linnaean Plant Name Typification Project.: http://www.nhm.ac.uk/research–curation/research/projects/linnaean–typification/database/; the Linnean Collections.: http://www.linnean–online.org/).

The determination of the number of chromosomes and the description of the species karyotype were carried out by light microscopy. The seeds were germinated on wet filter paper in Petri dishes in the laboratory (21°C). The karyological investigations were made on the mitotic metaphases of the meristematic cells from root tips. The root tips were pretreated in 0.4% colchicines solution for 2 h; fixed in fluid 3:1 alcohol and glacial acetic acid for at least 2 h at room temperature. After hydrolysis in HCl 1 N for 10–15 min at 60°C the root tips were stained in Schiff reagent at 1.5 h. Then the root tips were squashed on a glass slide with 45% acetic acid. For all chromosome counts, a minimum of 10 plates were examined for each taxon. The stained roots were washed in warm water and placed on a glass slide. The colored tip of the rootlet (0.5–1.0 mm) was crushed in 45% acetic acid under a coverslip. After a preliminary examination of the smears under a microscope, the preparations were placed in butyl alcohol for 5 min, then in xylene for 5 min, and were placed in Canadian balsam. The karyological investigation was based on the samples of species of the genus Geranium (Geraniaceae) collected by R. Adamyan, K. Tamanyan, G. Fayvush during expeditions in 2003–2010 to the different regions of Armenia. Herbarium material of the Institute of Botany of National Academy of Sciences (ERE) and Yerevan State University (EREU) was studied.

Results and Discussion. The genus Geranium L. is represented mainly by annuals and perennials herbs. In general, the genus is characterized by simple, palmately dissected, petiolate or sessile leaves, usually with stipules. All species of geranium have stipules. They can be membranous or leathery in texture, brown or green in color, pubescent or glabrescent. Stipules are lanceolate in shape, oblong-
ovate, ovate and always with a pointed apex. In some cases, they can serve as a diagnostic species feature.

The inflorescence of geranium is of the sympodial type and is characterized as a cymoid or closed thyrsus, where directly under the flower at the top of the main axis, one or more lateral axes develop, repeating the structure of the main axis [4].

Pedicels can be two-flowered or one-flowered, erect or deflexed during bearing. The flower of representatives of the genus is actinomorphic, bisexual or less often unisexual. The perianth consists of 5 sepals and 5 petals. Sepals are usually with 3 or 5 (7) veins, membranous margin and awn at apex. Petals are integral or emarginate at apex, with or without short clawed, pubescent or without pubescence at the base. There are 10 stamens in two circles, all with anthers. The stamens of the outer circle are shorter and opposite of the petals, while the stamens of the inner circle are longer and alternate with the petals.

Fruits in geranium syncarpic consists of 5 mericarps, the cavities of the nests of which gradually narrow upwards, forming a column (“beak”), specific to Geraniaceae family. There are five nectars alternating with petals. The sterigma of the genus Geranium develops from the top syncarp pentamer ovary and is considered by some authors to be a fractional box [5–7]. The mature fruit is divided into 5 single-seeded medicarpies hanging on the top of the central column (the preserved column) [8]. Mature seed in geranium is without endosperm or with a thin layer of endosperm [9]. The embryo fills completely inside the seed. The cotyledons are large, usually flat, folded and with edges on top of each other. They perform a storing function and contain oil and aleurone [10] (Tab. 1).

Table 1

Representative features of the genus Geranium

| Species          | Character of pubescent       | Length of awn sepals, mm | Degree of petal marginate | Carpels surface | Form of leaves plates | Seed surface       |
|------------------|------------------------------|--------------------------|----------------------------|-----------------|-----------------------|--------------------|
| *G. sanguineum*  | long white deflexed pilose   | 3                        | emarginate                 | not deep pilose | roundish-nodulated    | line-dotty         |
| *G. columbinum*  | tooth, upward eglandular pilose | 3                        | entire                     | smooth, without wrinkled pressed pilose | rounded or nodulated | with pits         |
| *G. dissectum*   | glandular pilose             | 0.5–2.0                  | emarginate                 | without wrinkled glandular pilose          | rounded or nodulated | with pits         |
| *G. ibericum*    | long and soft pilose         | 1.5–2.5                  | deeply emarginate with barb| smooth pilose  | roundish-pentagonal   | –                  |
| *G. montanum*    | long white remoted or deflexed pilose | 2–3                      | deeply emarginate          | thick pilose   | pentagonal            | –                  |
| *G. platypetalum*| long eglandular and short glandular pilose | 3–4                      | deeply, narrowly emarginate| pressed upward pilose | broadly wedge-shaped segments | dotty             |
There are 20 species of the genus *Geranium* growing in the flora of Armenia, and karyologically were investigated following species: *G. columbinum*, \(2n=18\) (sect. *Columbinum*); *G. ibericum*, \(2n=28\), *G. sylvaticum*, \(2n=28\) (sect. *Geranium*); *G. lucidum*, \(2n=40\) (sect. *Robertiana*); *G. pyrenaicum*, \(2n=26\); *G. pussillum*, \(2n=26\); *G. molle*, \(2n=26\); *G. rotundifolium*, \(2n=26\); *G. divaricatum*, \(2n=26\) (sect. *Rotundifolia*). According to the literature data, on the chromosome numbers of the species of the genus *Geranium*, presented in Tab. 1, the different basic chromosome numbers \(x=9, 10, 11, 12, 14, 15, 16, 17, 23, 25\) is revealed.

**G. columbinum** L., \(2n=18\). The species is karyologically well studied. According to the literature data, only the diploid cytorace is characteristic for *G. columbinum* with \(2n=18\) [3, 11–13]. Our material also revealed a diploid cytorace for this species \(2n=2x=18\) with basic chromosomes number \(x=9\). The karyotype of

| Table 1 Continuation |
|----------------------|
| **G. sylvaticum**    | remoted pilose  | 3 | entire or weakly emarginate | pressed pilose, smooth | roundish-nodulated | thinly-dotty |
| **G. ruprechtii**    | pressed pilose  | 3–4 | entire or weakly emarginate | – | rounded or nodulated | – |
| **G. robertianum**   | remoted pilose  | 5 | entire | reticulated wrinkled, glabrous | pentagonal | thinly-dotty |
| **G. lucidum**       | glabrous       | 0.2–0.5 | entire | reticulated wrinkled | rounded | smooth |
| **G. palustre**      | tooth or glandular pilose | 2–3 | entire | pilose, without idges | fivefold | smooth |
| **G. collinum**      | pressed sometimes glandular pilose | 0.1–0.2 | entire | smooth pilose | rounded | – |
| **G. albanum**       | long remoted pilose | 1 | emarginate | scallop-thickened outgrowths | nodulated | rounded | – |
| **G. pyrenaicum**    | long eglandular and short glandular pilose | 0.1–0.3 | deeply, narrowly emarginate | upward pilose | nodulated | rounded | glabrous |
| **G. pussillum**     | eglandular and glandular pilose | 0.1–0.2 | emarginate | pressed pilose, without wrinkled | rounded or roundish-nodulated | smooth |
| **G. molle**         | soft short pilose | 0.1–0.2 | deeply emarginate | glabrous, transversely wrinkled | roundish-nodulated | smooth |
| **G. rotundifolium** | soft uppermost glandular pilose | 0.2–0.5 | entire | smooth | roundish-nodulated with pits |
| **G. divaricatum**   | glandular and soft remoted eglandular pilose | 1.5 | emarginate | transversely wrinkled | pinnate-fivelod | smooth |
| **G. tuberosum**     | short and thick pilose | 1 | weakly emarginate | smooth pilose | nearly rounded | smooth |
| **G. linearilobum**  | short declinate pilose | 1 | weakly emarginate | smooth | nearly rounded | smooth |
G. colombinum is asymmetric, consisting of 2 pairs of submetacentric and 7 pairs of metacentric chromosomes. Karyotype formula is: $2n=18=4SM+14M$.

G. ibericum Cav., $2n=28$. The species is poorly studied karyologically. According to the literature data, for the European species G. ibericum, mainly the tetraploid cytotype was found $2n=4x=56$ [14, 15], in addition, a diploid cytotype is known for this species $2n=28$ [16]. On our material diploid cytotype was also identified for this species $2n=2x=28$ with basic chromosomes number $x=14$. The karyotype of G. ibericum is asymmetric, consisting of 7 pairs of metacentric, 4 pairs of submetacentric and 3 pairs of chromosomes with satellites: 2 pairs of metacentric and 1 pairs of submetacentric satellite chromosomes. Karyotype formula is: $2n=28=14M+8SM+4MS+2SMS$.

G. sylvaticum L., $2n=28$. The species is karyologically well studied. According to the literature data, mainly the diploid cytotype is characteristic for G. sylvaticum, $2n=28$, with basic chromosomes number $x=14$ [16–19]. For the Belarusian material S.A. Dmitrieva gives the number of chromosomes $2n=24$ [20]. Our material also revealed a diploid cytotype for this species $2n=2x=28$ with basic chromosomes number $x=14$. The karyotype of G. sylvaticum is asymmetric, consisting of 6 pairs of metacentric, 5 pairs of submetacentric and 3 pairs of chromosomes with satellites: 2 pairs of metacentric and 1 pairs of submetacentric chromosomes with satellites. Karyotype formula is: $2n=28=12M+10SM+4MS+2SM^S$.

G. lucidum L., $2n=40$. The species is karyologically well studied. According to the literature data, for the species G. lucidum, in most cases, di- and tetraploid cytotypes were found $2n=20, 40$ [12, 13, 17, 18, 21–23]. In addition, for G. lucidum, a hexaploid cytotype $2n=60 (6x)$ is given on Indian populations of this species [24], and for Belarusian populations, an octoploid chromosome number is also known, $2n=80$ [25]. For European populations of this species, chromosome numbers $2n=40–44$ were exhibited [13, 16]. In the studied specimens of the species G. lucidum, we found tetraploid cytotype $2n=4x=40$ with basic chromosomes number $x=10$. Karyotype of this species is asymmetric, consisting of 5 pairs of metacentric, 12 pairs of submetacentric and 3 pairs of submetacentric chromosomes with satellites. Karyotype formula is: $2n=40=10M+24SM+6SM^S$.

G. pyrenaicum Burm. F., $2n=26$. The species is karyologically well studied. According to the literature data, for the species G. pyrenaicum mainly the diploid cytotype is characteristic for G. pyrenaicum $2n=26$ [11, 12, 16, 18, 21, 26–30], only for Indian populations is given $2n=2x=20$ chromosomes number with basic chromosomes number $x=10$ [31], and for European – $2n=28$ with basic number $x=14$ [32]. In the studied specimens of the species G. pyrenaicum also revealed a diploid cytotype $2n=2x=26$ with basic chromosomes number $x=13$, which confirms the previously presented numerous data. Karyotype of this species is asymmetric, consisting of 8 pairs of metacentric, 3 pairs of submetacentric chromosomes and on 1 pairs of meta- and submetacentric chromosomes with satellites. Karyotype formula is: $2n=26=16M+6SM+2M^S+2SM^S$.

G. pussilum L., $2n=26$. The karyology of this species has been fairly well investigated. According to the literature data, for the species G. pussilum mainly the diploid cytotype is found $2n=26$ with basic chromosomes number $x=13$ [11, 13, 19, 26, 33, 34]. In the studied specimens of the species G. pussilum we found diploid cytotype $2n=2x=26$ with basic chromosomes number $x=13$, which confirms the
previously presented numerous data. The chromosomes number is given for the first
time for the flora of Armenia. Karyotype of this species is asymmetry. Diploid set
of chromosomes consists of 8 pairs of metacentric, 3 pairs of submetacentric
chromosomes and 2 pairs of metacentric chromosomes with satellites. Karyotype
formula is: $2n=26=16M+6SM+4M^3$.

**G. molle** L., $2n=26$. The karyology of this species has been fairly well
investigated. According to the literature data, for the species **G. molle** mainly the
diploid cytotype is found $2n=26$ [12, 13, 35–37]. In the studied specimens of the
species **G. molle** we found diploid cytotype $2n=2x=26$ with basic chromosomes
number $x=13$, which confirms the previously presented numerous data. Karyotype
of this species is asymmetric, consisting of 3 pairs of metacentric, 8 pairs of sub-
metacentric chromosomes and 1 pairs of metacentric and 1 pairs of submetacentric
chromosomes with satellite. Karyotype formula is: $2n=26=6M+16SM+2M^3+2SM^3$.

**G. rotundifolium** L., $2n=26$. The karyology of this species has been fairly
well investigated. According to the literature data, mainly the diploid cytotype is
characteristic for the species **G. rotundifolium** $2n=26$ [12, 18, 21, 34, 36]. In the
studied specimens of this species we also found diploid cytotype $2n=2x=26$ with
basic chromosomes number $x=13$. Karyotype of this species is asymmetric,
consisting of 8 pairs of metacentric, 3 pairs of submetacentric chromosomes and
2 pairs meta-, 2 pairs submetacentric chromosomes with satellites. Karyotype
formula is: $2n=26=16M+6SM+2M^3+2SM^3$.

**G. divaricatum** Ehrh., $2n=26$. The species is poorly studied karyologically.
According to the literature data, for the Slovakian **G. divaricatum** species mainly
was found chromosomes number $2n=26$ with basic number $x=13$ [11], for the Euro-
pean species is given diploid cytotype $2n=28$ with basic number $x=14$ [13, 16, 38].
For our material also revealed a diploid cytotype for this species $2n=2x=26$ with
basic chromosomes number $x=13$. The chromosomes number is given for the first
time for the flora of Armenia. Karyotype of this species is asymmetry, consisting of
8 pairs of metacentric, 4 pairs of submetacentric chromosomes and 1 pairs of meta-
centric chromosomes with satellites. Karyotype formula is: $2n=26=16M+8SM+2M^3$.

The representatives of genus *Erodium* are annual, biennial or perennial herbs.
The stems of the majority are ascending, simple or scarcely, or else they have rosette
forming. Often there is a distinction between stem and basal leaves. Basal leaves are
ovate, shortly 3–5 lobed, crenate or crenate-dentate. Leaf size and petiole length are
steadily declining over the stem. Upper cauline leaves are usually binary and
opposite, more acutely toothed or crenate-lobed, pinnatifid or dissected with
stipules. The inflorescence is umbel with bracts, sometimes reduced to 1–2 flowers.
The flowers of genus representatives are actinomorphic and consist of five sepals,
five petals and five nectaries. Dorsal part of sepals is covered with 3 or 5 (7) veins
and awn at apex. Petals are integral or emarginate at apex, with or without short claw,
pubescent or without pubescence at the base. Five fertile stamens and five
staminodes enclose the gynoecium. The fruit consists of 5 carpels and has a long
beak-like extension. Mericarps hairy with stiff separate from the stylar column
(rostrum) and awn spirally twisted towards the base, falcate and not twisted above,
stiffly plumose along the adaxial surface, reactive to atmospheric humidity thus
working the mericarp into the soil [5, 39]. The seeds are differentiated with elongated shape (Tab. 2).

| Species                  | Character of pubescent | Length of awn sepals, mm | Degree of petalse marginate | Length of carpels beak, cm | Form of leaves plates |
|--------------------------|------------------------|--------------------------|----------------------------|---------------------------|----------------------|
| E. oxyrrhynchum          | small pressed pilose   | 3                        | entire                     | 8.0–9.5                   | oblong ovate         |
| E. armenum               | longe white declinate pilose and small glandular pilose | 0.5–1.0                  | entire                     | 8                         | oblong               |
| E. sosnowskianum         | small glandular and longe eglandular pilose | without own              | entire                     | 3.5–4.0                   | oblong               |
| E. hoefftianum           | glandular pilose       | 3                        | weakly emarginate          | 6–8                       | oblong ovate         |
| E. ciconium              | eglandular lamellar and weakly glandular pilose | 0.2–0.5                  | weakly emarginate          | 7–10                      | ovate oblong         |
| E. cicutarium            | lamellar or glandular pilose | 0.2–0.5                  | entire                     | 4–5                       | oblong               |

According to the literature data, on the chromosome numbers of the species of the genus Erodium, the different basic chromosome numbers $x=9, 10, 14, 18, 19$ is revealed, where the basic number of chromosomes $x=10$ predominates. Unfortunately, due to the poor germination of seeds, difficulties arose for the karyological study of species of the genus Erodium in the Armenian flora.

**Conclusion.** For genera and species differentiation we used main morphological characteristics of Geraniaceae family. The structure of fruits is distinctive for Geranium and Erodium genera differentiation. The mature fruits beak is bald and bends in Geranium genus, while it is hairy and spiral twisted in Erodium. The leaves of Geranium representatives are finger–lobed or divided, the length not exceeding its width, while leaves of Erodium are dentate- or crenate-lobed, pinnately divided or dissected, the length always exceeding its width. The degree of leaves dissection is a good character for the sectional division. Diagnostic characters for species of the family Geraniaceae are the degree of hairiness, types of hair, their position and length; leaf morphology and their location on the stem; shape, texture, color and pubescence of stipules; pubescence and position of pedicels; size, pubescence and number of veins on the sepals, as well as the size of the awn; the size of the clawed or petals; color of stamens; the nature of the valves of the fetus, the size of the elongated part of the beak; the nature of the surface of the seeds.

The karyological research of the genus Geranium, growing in Armenian flora, has revealed the following: for 8 species we discovered diploid cytoraces – G. columbinum $2n=18$ (sect. Columbinum), G. ibericum $2n=28$, G. sylvaticum $2n=28$ (sect. Geranium), G. pyrenaicum $2n=26$, G. pussillum, $2n=26$, G. molle, $2n=26$, G. rotundifolium, G. divaricatum, $2n=26$ (sect. Rotundifolia) $2n=26$ with different basic chromosome numbers $x=9, 13, 14$. For the species G. lucidum (sect. Robertiana)
a tetraploid cytortace $2n=40$ was discovered with the basic chromosome number $x=10$. The different basic chromosome numbers $x=9, 10, 13, 14$ (Tab. 3) shows up in the genus *Geranium*.

| $x$ | 9  | 10 | 13 | 14 |
|-----|----|----|----|----|
| $2x$ | 18 | –  | –  | –  |
| $3x$ | –  | –  | 26 | 28 |
| $4x$ | –  | –  | 40 | –  |

The species of the section *Rotundifolia*, growing on the territory of Armenia (*G. pyrenaicum*, $2n=26$; *G. pussilum*, $2n=26$; *G. molle*, $2n=26$; *G. rotundifolium*, $2n=26$; *G. divaricatum*, $2n=26$), are characterized with the basic main chromosome number of $x=13$.

The main basic chromosome number of $x=9$ is typical for the species of the section *Columbinum* growing on the territory of Armenia (*G. columbinum*, $2n=18$).

The main basic chromosome number of $x=10$ is typical for the species of the section *Robertiana*, growing on the territory of Armenia (*G. lucidum*, $2n=40$).

The main basic chromosome number of $x=14$ is typical for the species of the section *Geranium*, growing on the territory of Armenia (*G. ibericum*, $2n=28$; *G. sylvaticum*, $2n=28$).

Asymmetric karyotype is observed in all the karyologically investigated species of the genus *Geranium*.

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МОРФОЛОГИЧЕСКОЕ И КАРИОЛОГИЧЕСКОЕ ИССЛЕДОВАНИЕ ВИДОВ СЕМЕЙСТВА GERANIACEAE JUSS. ФЛОРЫ АРМЕНИИ

В связи с таксономической обработкой представителей сем. Geraniaceae флоры Армении проведен анализ диагностических признаков. При идентификации видов обычно учитывается строение корневой системы, жизненная форма растения, морфология листа, строение цветка, соцветие, строение плодов. В результате кариологического исследования видов рода Geranium (сем. Geraniaceae), произрастающих в Армении, для 8 видов обнаружена диплоидная и тетраплоидная циторасса. В роде Geranium вырисовывается анеуплоидный ряд основных хромосомных чисел \( x = 9, 10, 13, 14 \).