Caryologia
International Journal of Cytology, Cytosystematics and Cytogenetics

Firenze University Press
www.fupress.com/caryologia

OPEN ACCESS

Citation: E. Mitrenina, M. Skaptsov, M. Kutsev, A. Kuznetsov, H. Ikeda, A. Erst (2020) A new diploid cytotype of *Agrimonia pilosa* (Rosaceae). *Caryologia* 73(1): 67-73. doi: 10.13128/caryologia-170

Received: February 19, 2019

Accepted: February 23, 2020

Published: May 8, 2020

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.

A new diploid cytotype of *Agrimonia pilosa* (Rosaceae)

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Abstract. A new diploid cytotype of *Agrimonia pilosa* Ledebour (Rosaceae) collected in China has been revealed. Karyotype formula is $2n = 2x = 16 = 14m + 2sm$. Previously, chromosome numbers in *A. pilosa* established by other researchers were $2n = 28$; 56; 70 with basic chromosome number $x = 7$. All the other members of genus *Agrimonia* Linnaeus have the same basic chromosome number. In the meanwhile, some members of fam. Rosaceae have different basic chromosome numbers: $x = 8$ (e.g., in genera *Amygdalus* L., *Aphanes* L., Cerasus Mill., etc.), $x = 9$ (e.g., in genera *Adenostoma* Hook. & Arn., *Chamaebatia* Benth., etc.), $x = 17$ (e.g., in genera *Amelanchier* Medik., *Chaenomeles* Lindl., etc.). We suppose that the new basic chromosome number $x = 8$ was revealed in *Agrimonia pilosa* collected in China.

Keywords. *Agrimonia pilosa* Ledebour, Rosaceae, chromosomes, karyotype, new cytotype, flora of China.

1. INTRODUCTION

Genus *Agrimonia* Linnaeus (fam. *Rosaceae*, subfam. *Rosoideae*) comprises 15 to 25 species and some naturally occurring hybrids distributed mainly in temperate regions throughout Europe, Asia, North America, Central America, the West Indies, southern South America, and the Southern Africa (Li *et al*. 2003; Chung 2008; Kline and Sørensen 2008; Angelo and Boufford 2012). The genus belongs to the tribe *Sanguisorbeae* DC divided into two subtribes – *Agrimoniinae* J. Presl and *Sanguisorbeae* Torr. & A. Gray. The first subtribe, along with genus *Agrimonia*, includes genera *Aremonia* Neck. ex Nestl., *Hagenia* J. F. Gmel., *Leucosidea* Eckl. & Zeyh., *Spenceria* Trimen (Potter *et al*. 2007). Last four genera are monotypic endemics (Chung 2008; Chung *et al*. 2012). Species of the subtribe display basic chromosome number $x = 7$ and different levels of ploidy ($2x$, $4x$, $6x$, $8x$, $10x$, $12x$) correlating with geographic distribution patterns (Chung 2008; Rice *et al*. 2015).

Several species in *Agrimonia* (e.g., *A. pilosa*, *A. eupatoria*) are used for medicinal purposes. They have been reported to possess antibacterial (Muruzović *et al.* 2016), antiviral (Kwon *et al.* 2005), antitumor (Miyamoto *et al.* 1987; Tang *et al.* 2017), diuretic (Giachetti 1986) and antidiabetes properties (Swanston-Flatt *et al.* 1990; Kuczmannová *et al.* 2016), antioxidant (Chen and Kang 2014; Muruzović *et al.* 2016), immunomodulating (Bukovsky and Blanarik 1994), hepatoprotective (Park *et al.*, 2004) and other effects.

In flora of China, the genus comprises following species: *Agrimonia coreana* Nakai, *Agrimonia eupatoria* Linnaeus subsp. *asiatica* (Juzepczuk) Skalický, *Agrimonia nipponica* Koidzumi var. *occidentalis* Skalický ex J. E., *Agrimonia pilosa* Ledebour (*Agrimonia pilosa* var. *pilosa* and *Agrimonia pilosa* var. *nepalensis* (D. Don) Nakai) (Li *et al.*, 2003).

In the current study, karyotype analysis of *A. pilosa* collected in China (Figure 1) has been conducted. A new diploid cytotype $2n = 16$ and a new probable basic chromosome number $x = 8$ for the genus *Agrimonia* L. were revealed.

Combination of chromosome investigation with morphological methods, molecular genetics methods and scanning electron microscopy gives possibility to obtain essential data to reach conclusions on plants systematics and phylogeny.

Figure 1. *Agrimonia pilosa* Ledebour (Beijing, China).

2. MATERIALS AND METHODS

Seeds of *A. pilosa* for cytological study and herbarium specimens were collected in China, Beijing, Yun Xiu Gu Forest park, Rocky ledges (40°60'N; 117°41'E, 22 July 2016; collectors: Erst A.S., Erst T.V., Lian L., Bing L., Shi C) and were collected in Russia, West Sibiria, Tomsk, southern edge of the city, inundation meadow (56°47'N; 85°03'E, 1 Sep. 2017; collector: Mitrenina E. Yu.). All herbarium materials are deposited in Novosibirsk (NS).

2.1. Karyotype analysis

Mitotic metaphase chromosomes in root tips of seedlings were studied. Seeds were grown at Petri dishes with wet sand at room temperature after cold stratification at 3–4° C during 4 months. Newly formed roots about 1.0–1.5 cm long were pretreated in a 0.2% colchicine solution during 2 hours at room temperature. Fixation was carried out in a mixture of absolute ethanol and glacial acetic acid (3 : 1). Root tips were stained in 1% aceto-haematoxylin, and the squashing method was employed for investigating of karyotype (Smirnov 1968).

Chromosomes were counted in 127 mitotic cells of 5 *A. pilosa* seedlings collected in China and in 25 mitotic cells of 5 *A. pilosa* seedlings collected in Russia. Mitotic metaphase chromosome plates were observed by microscope Primo Star (Carl Zeiss, Germany) and photographed by microscope AxioImager A.1 (Carl Zeiss, Germany) with software AxioVision 4.7 (Carl Zeiss, Germany) and CCD-camera AxioCam MRc5 (Carl Zeiss, Germany) at 1000× magnification at Laboratory for Ecology, Genetics and Environmental Protection ("Ecogene") of National Research Tomsk State University. For karyotyping, the software KaryoType (Altinordu *et al.* 2016) was used, and for figures, the software Adobe Photoshop CS5 (Adobe Systems, USA) and Inkscape 0.92 (USA) was used. The measurements were performed on 10 metaphase plates. For analysis of karyotype, the nomenclature of Levan, Fredgam, and Sandberg (1964) has been used.

2.2. Flow cytometry

Flow cytometry with propidium iodide (PI) staining was implemented to determine relative DNA content. At least 10 seeds from each plant were taken for this study. Each seed was analysed separately. Seed buffer (Matzk *et al*. 2001) was used for nuclei extraction. Seeds was squashed by porcelain pestle and chopped with a sharp razor blade in the nuclei extraction buffer. The samples were filtered through 50-μm nylon membrane into a sample tube. Flow cytometry with Partec CyFlow PA revealed the data on isolated nuclei fluorescence (Partec, GmbH) using the laser 532 nm wavelength, while logarithmic fluorescence data representation (logarithmic scale) was used to record the signals. To calculate the mean of peak, at least 1000 nuclei peaks with less than 2.5% CV indicator values were used. The final data did not exceed the DNA content of the mean sample by more than 3% (Kubešová *et al*. 2010). As an external standard was used *Euryops chrysanthemoides* (DC.) B. Nord, 2C = 2.70 pg, and internal standard *Glycine max* 'Polanka', 2C = 2.50 pg (Doležel *et al*. 1994; Skaptsov *et al*. 2016). We used the Statistica 8.0 software (StatSoft Inc.), Flowing Software 2.5.1 (Turku Centre for Biotechnology) and CyView software for the flow cytometer data analysis (Partec, GmbH), and for the analysis of our results. The possible effect of secondary metabolites on the binding of the intercalating dye was evaluated by co-grinding in a nuclei extraction buffer of the samples and *Allium fistulosum* L. leaves. The resulting preparation was investigated three times within 10 minutes. In the absence of variations in the average values of the detection channels of the *A. fistulosum* peak, it was believed that no effect was detected.

Flow cytometry performed at the Laboratory of Bioengineering of South-Siberian Botanical Garden, Altai State University.

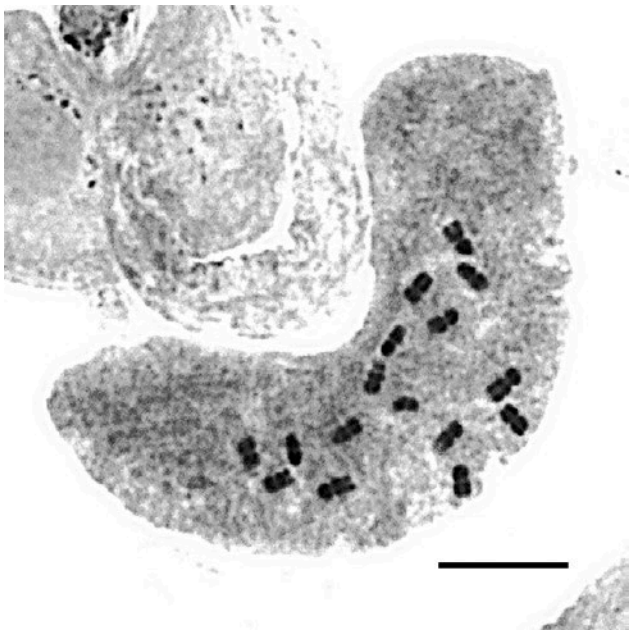

Figure 2. Mitotic metaphase chromosomes of *Agrimonia pilosa* Ledebour (Beijing, China), 2*n* = 16. Scale bar = 10 μm.

3. RESULTS

Karyotype analysis of *A. pilosa* collected in China, Beijing has been conducted. All 127 investigated cells in 5 seedlings had diploid chromosome number 2*n* = 16 (Figure 2). By the software KaryoType (Altinordu *et al*. 2016) morphometric chromosome analysis (total chromosome length, short and long chromosome arms length, arm ratio) has been conducted (Table 1). Chromosome length ranged from 1.87 ± 0.17 μm to 2.14 ± 0.18 μm. Arm ratio varied from 1.03 to 1.81. Chromosomes were classified into two groups: seven pairs with median centromeric position (metacentric chromo-

Table 1. Karyotype parameters of *Agrimonia pilosa* Ledebour, China (2*n* = 16).

Chromosome pair	Total length, μm, ± SD	Long arm, μm, ± SD	Short arm, μm, ± SD	Arms ratio (long/short)	Chromosome type
1	2.14 ± 0.18	1.29 ± 0.12	0.85 ± 0.07	1.52	m
2	2.10 ± 0.24	1.10 ± 0.11	1.00 ± 0.13	1.10	m
3	2.04 ± 0.23	1.12 ± 0.13	0.92 ± 0.12	1.22	m
4	2.02 ± 0.24	1.15 ± 0.26	0.87 ± 0.11	1.32	m
5	1.95 ± 0.29	1.06 ± 0.17	0.89 ± 0.13	1.19	m
6	1.88 ± 0.17	1.05 ± 0.22	0.83 ± 0.08	1.27	m
7	1.87 ± 0.17	0.95 ± 0.08	0.92 ± 0.09	1.03	m
8	2.11 ± 0.27	1.36 ± 0.17	0.75 ± 0.10	1.81	sm

Notes: m – metacentric chromosome; sm – submetacentric chromosome; ± SD – mean length ± standard deviation.

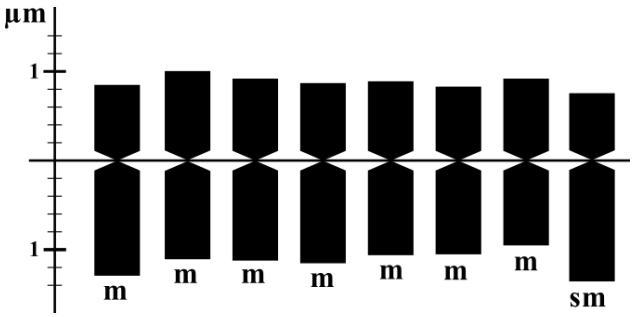

Figure 3. Idiogram of *Agrimonia pilosa* Ledebour (Beijing, China), $2n = 16$. m – metacentric chromosome, sm – submetacentric chromosome.

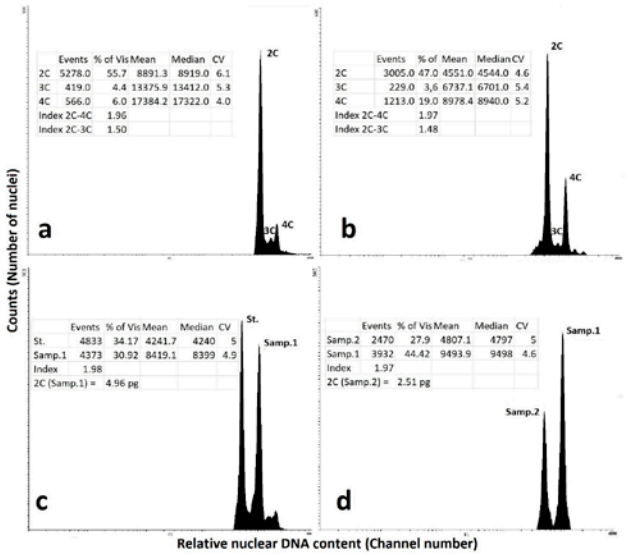

Figure 4. Flow cytometry histograms: **a** – *Agrimonia pilosa* (Tomsk, Russia); **b** – *Agrimonia pilosa* (Beijing, China); **c** – *Agrimonia pilosa* (Tomsk, Russia), Samp. 1 with internal standard (*Glycine max* (L.) Merr.), St.; **d** – *Agrimonia pilosa* (Tomsk, Russia), Samp. 1 with *Agrimonia pilosa* (Beijing, China), Samp. 2.

somes, m; arm ratio 1–1.7), and one pair with sub-median centromeric position (submetacentric chromosomes, sm; arm ratio 1.7–3.0). Some metacentric pairs were low-differentiated. They had almost equal length and arm ratio. Karyotype formula is $2n = 2x = 16 = 14m + 2sm$ (Figure 3). Karyotype asymmetry degree (Stebbins 1971): 1A. Secondary constrictions in 1–2 metaphase chromosome pairs were revealed. Nucleolus number observed in mitotic interphase were 1–2 per cell.

Chromosome counting in *A. pilosa* collected in Russia, Tomsk has revealed typical octaploidic cytotype for the species: $2n = 8x = 56$. By the flow cytometry method, we have found out relative DNA content in two agrimonies: 2C = 2.51 pg in *A. pilosa* (China) with $2n = 16$, and 2C = 4.96 pg in *A. pilosa* (Russia) with $2n = 56$ (Figure 4).

4. DISCUSSION

According to the data of Chromosome Counts Database (Rice *et al.* 2015), Index to Plant Chromosome Numbers and other learned treatise (Iwatsubo *et al.* 1993; Chung 2008; Angelo and Boufford 2012; Kumar *et al.* 2014), diploid chromosome numbers in *Agrimonia* are known as 28; 42; 56; 70 и 84 (Table 2). The basic chromosome number in the genus $x = 7$. Currently, within *Agrimonia* only polyploids have been reported. As long as the lowest ploidy levels reported among species of *Agrimonia* are tetraploids, the lineage appears to have an ancient origin where diploids have gone extinct (Chung 2008). Such a basic chromosome number is common for many members of fam. *Rosaceae* (e.g., genera *Geum* L., *Potentilla* L., *Rosa* L., etc.). At the same time, there are other basic chromosome numbers in *Rosaceae*: $x = 8$ (e.g., in genera *Amygdalus* L., *Aphanes* L., *Cerasus* Mill., *Exochorda* Lindl, *Padus* Mill., *Prunus* L.), $x = 9$ (e.g., in genera *Adenostoma* Hook. & Arn., *Chamaebatia* Benth., *Holodiscus* Maxim.), $x = 17$ (e.g., in genera *Amelanchier* Medik., *Chaenomeles* Lindl., *Kageneckia* Ruiz & Pav.) (Rice *et al.* 2015). Conventionally, subfamily classification was based on a combination of basic chromosome numbers and fruit types (Chung *et al.* 2012). Other genera belonging to subtribe *Agrimoniinae*,

Table 2. Chromosome numbers in the genus *Agrimonia* L. (Chung 2008; Angelo and Boufford 2012; Rice *et al.* 2015).

Species	Chromosome numbers
Agrimonia coreana Nakai	24; 28
Agrimonia eupatoria L.	28; 42; 56; 70; 84
Agrimonia grandis Andrz. ex C. A. Mey.	42
Agrimonia gryposepala Wallroth	56
Agrimonia incisa Torr. & A. Gray	28
Agrimonia japonica (Miq.) Koidz.	56
Agrimonia nipponica Koidz.	28
Agrimonia parviflora Aiton	28
Agrimonia pilosa Ledeb.	28; 56; 70
Agrimonia x *nipponica-pilosa* Murata	42
Agrimonia procera Wallr.	56
Agrimonia pubescens Wallroth	28
Agrimonia repens L.	28
Agrimonia rostellata Wallroth	28
Agrimonia striata Michx.	28; 56

disregarding *Agrimonia*, have following chromosome numbers and ploidy: *Aremonia* – $2n = 5x = 35$ and $2n = 6x = 42$, *Hagenia* – $2n = 6x = 42$, *Leucosidea* and *Spenceria* – $2n = 2x = 14$ (Ikeda *et al*. 2006; Chung *et al*. 2012; Rice *et al*. 2015).

Previously, a karyotype of *A. pilosa* var. *japonica* was examined by Iwatsubo *et al*. (1993). All studied plants had $2n = 56$. Chromosomes at metaphase ranged 1.2–2.5 μm in length and 1.0–2.5 in arm ratio. These were classified into two groups: 21 metacentric pairs, and seven submetacentric pairs. One submetacentric pair had a satellite on the short arm. According to other scientific data, somatic chromosome number of *A. pilosa* are $2n = 28$ and $2n = 70$ (Table 2).

We had revealed a new diploid cytotype in *A. pilosa* collected in China with $2n = 16$. Apparently, these plants exhibit diploid karyotype and new basic chromosome number $x = 8$ for the genus and subtribe. That chromosome number $2n = 16$ was determined in all 127 investigated root meristematic cells. We suppose there were no B chromosomes for diploid cytotype $2n = 14$. Dysploidy arising from chromosomes fusions (Escudero *et al*. 2014) is also unlikely, having our data on chromosomes length and morphology are relevant to the results previously obtained on *A. pilosa* with $2n = 56$ by Iwatsubo *et al*. (1993). We suppose that a haploidization of genome took place in *A. pilosa* specimen collected in China. According to classification developed by Kimber and Riley (1963), this event relates to aneupolyhaploidia, that is haploidization of polyploid form associated with aneuploidy.

In addition to karyotype`s divergence from typical polyploid *A. pilosa* with $2n = 56$, the investigated herbarium specimen exhibits reduced dimensions, fruits, and seeds. This corresponds with the revealed less ploidy level of the plant because polyploidy is followed by «gigas»-effect (Ramsey and Ramsey 2014). We do not exclude the possibility that this specimen could be related to a new taxon.

Our flow cytometry studies revealed that C-values in two investigated *A. pilosa* differ at a factor of two, approximately. This result was unexpected to us due to the fact that the number of chromosomes didn`t correlate with relative DNA content in two examined agrimonies. Unfortunately, we had no *A. pilosa* specimen with chromosome number $2n = 28$ to determine relative DNA content and to compare it with data obtained.

The sizes of the monoploid genome were found to be equal 1Cx = 1.25 pg for samples with $2n = 16$, and 1Cx = 0.62 pg for samples with $2n = 56$ which indicates a significantly more ancient origin of diploid populations, according to the genome downsizing theory (Leitch, Bennett 2004). Due to other studies reports, DNA loss in polyploid series is usually at the level of 15.4% (Zenil-Fergusson *et al*. 2016), whereas in our case, such a significant decrease may indicate complex molecular-genetic processes and DNA loss during the evolution of the *Agrimonia pilosa* genome. Studies of many eukaryotic genomes show that noncoding regions of DNA can be lost in the polyploidization process (Shaked *et al*. 2001). Some cytological studies show a loss of heterochromatin, whole chromosomes or their segments after polyploidization (Gustafson and Bennett 1982; Song *et al*. 1995; Chen and Ni 2006; Xiong *et al*. 2011). Thus, our study is correlated with the idea that the reduction of the genome is a frequent biological phenomenon.

More detailed investigation of the herbarium specimen of *A. pilosa* collected in China by molecular cytogenetics and molecular genetics methods with morphological analysis can elucidate the problem of evolution of the genus *Agrimonia*.

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the author.

FUNDING

The research was supported by the Scientific Programme AAAA-A17-117012610055-3 of the Central Siberian Botanical Garden, of SB RAS (field trip), "The Tomsk State University competitiveness improvement programme" under Grant No 8.1.09.2018 (karyotyping works); state assignment of the Altay State University under Grant No FZMW-2020-0003 (genome size works). We would like to thank the reviewers for their valuable comments and suggestions. Authors are grateful to Roman Annenkov for preparing Figures 2 and 3.

REFERENCES

Angelo R, Boufford DE. 2012. Atlas of the flora of New England: Rosaceae. Phytoneuron. 81:1–56.

Altinordu F, Peruzzi L, Yu Y, He X. 2016. A tool for the analysis of chromosomes: KaryoType. Taxon. 65(3):586–592.

Bukovsky M, Blanarik P. 1994. Immunomodulative effects of ethanolic-aqueous extracts of Herba agrimoniae, Flos chamomillae and Flos calendulae cum calyce. Farm Obz. 63(4):149–156.

Chen L, Kang YH. 2014. Antioxidant activities of *Agrimonia pilosa* Ledeb: in vitro comparative activities of its different fractions. Korean J Plant Res. 27(6):642–649.

Chen ZJ, Ni Z. 2006. Mechanisms of genomic rearrangements and gene expression changes in plant polyploids. Bioessays. 28:240–252.

Chung KS. 2008. A systematic study of genus *Agrimonia* (Rosaceae). Doctoral Dissertation. University of Oklahoma, Norman.

Chung, KS, Hoang N, Elisens W, Un Oh B. 2012. Phylogenetic implication of seed coat sculpturing in subtribe Agrimoniinae (Rosaceae). Korean J Pl Taxon. 42(4):247–252.

Escudero M, Martín-Bravo S, Mayrose I, Fernández-Mazuecos M, Fiz-Palacios O, Hipp AL, Pimentel M, Jiménez-Mejías P, Valcárcel V, Vargas P *et al*. 2014. Karyotypic changes through dysploidy persist longer over evolutionary time than polyploid changes. PLoS ONE. 9(1):e85266.

Doležel J, Doleželova M, Novak FJ. 1994. Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*). Biol Plant. 36:351–357.

Giachetti D, Taddei E, Taddei I. 1986. Diuretic and urisurica of *Agrimonia eupatoria* L. Boll Soc Ital Biol Sper. 62(6):705–711.

Gustafson JP, Bennett MD. 1982. The effect of telomeric heterochromatin from *Secale cereale* on Triticale (x Triticosecale). I. The influence of the loss of several blocks of telomeric heterochromatin on early endosperm development and kernel characteristics at maturity. Can J Genet Cytol. 24:83–92.

Ikeda H, Hand K, Wu SK, Ohba H. 2006. A Revision of the genus *Spenceria* Trimen (Rosaceae). J Jpn Bot. 81(3):154–167.

Iwatsubo Y, Mishima M, Naruhashi N. 1993. Chromosome studies of Japanese *Agrimonia* (Rosaceae). Cytologia. 58:453–461.

Kimber G, Riley R. 2011. The relationships of the diploid progenitors of hexaploid wheat. Can J Genet Cytol. 5:83–88.

Kline GJ, Sørensen P. 2008. A Revision of *Agrimonia* (Rosaceae) in North and Central

America. Brittonia. 60(1):11–33.

Kubešová M, Moravcová L, Suda J, Jarošík V, Pyšek P. 2010. Naturalized plants have smaller genomes than their non-invading relatives: a flow cytometric analysis of the Czech alien flora. Preslia. 82:81–96.

Kuczmannová A, Balažová A, Račanská E, Kameníková M, Fialová S, Majerník J, Nagy M, Gál P, Mučaji P. 2016. *Agrimonia eupatoria* L. and *Cynara carduncu*lus L. Water infusions: comparison of anti-diabetic activities. Molecules. 21(5):564.

Kumar P, Rana PK, Himshikha, Singhal VK, Gupta RC. 2014. Chromosome numbers, characterization of chromosomal pairing during meiosis, origin and natural propagation in polyploidy cytotypes (4*x*, 5*x* and 6*x*) of *Agrimonia eupatoria* L. (Rosaceae) in Northwest Himalayas (India). Protoplasma. 251(4):781–795.

Kwon DH, Kwon HY, Kim HJ, Chang EJ, Kim MB, Yoon SK, Song EY, Yoon DY, Lee YH, Choi IS *et al*. 2005. Inhibition of hepatitis B virus by an aqueous extract of *Agrimonia eupatoria* L. Phytother Res. 19:355–358.

Leitch IJ, Bennett MD. 2004. Genome downsizing in polyploid plants. Biol J Linn Soc. 82(4):651–663.

Levan A, Fredgam K, Sandberg A. 1964. Nomenclature for centrometric position of chromosomes. Hereditas. 52:201–220.

Li CL, Ikeda H, Ohba H. 2003. *Agrimonia* L. In: Wu ZY, Raven PH, Hong DY, editors. Flora of China. Rosaceae, Vol. 9. Beijing, China: Science Press, pp. 384–387.

Matzk F, Meister A, Brutovská R, Schubert I. 2001. Reconstruction of reproductive diversity in *Hypericum preforatum* L. opens novel strategies to manage apomixis. Plant J. 26(3):275–282.

Miyamoto K, Kishi N, Koshiura R. 1987. Antitumor effect of agrimoniin, a tannin of *Agrimonia pilosa* Ledeb., on transplantable rodent tumors. Jpn J Pharmacol. 43(2):187–195.

Muruzović MŽ, Mladenović KG, Stefanović OD, Vasić SM, Čomić LR. 2016. Extracts of *Agrimonia eupatoria* L. as sources of biologically active compounds and evaluation of their antioxidant, antimicrobial, and antibiofilm activities. J Food Drug Anal. 24(3):539–547.

Park EJ, Oh H, Kang TH, Sohn DH, Kim YC. 2004. An isocoumarin with hepatoprotective activity in Hep G2 and primary hepatocytes from *Agrimonia pilosa*. Arch Pharm Res. 27(9):944–946.

Potter D, Eriksson T, Evans RC, Oh S, Smedmark JEE, Morgan DR, Kerr M, Robertson KR, Arsenault M, Dickinson TA *et al*. 2007. Phylogeny and classification of Rosaceae. Pl Syst Evol. 266(1–2):5–43.

Ramsey J, Ramsey TS. 2014. Ecological studies of polyploidy in the 100 years following its discovery. Philos Trans R Soc Lond B Biol Sci. 369(1648):1–20.

Rice A, Glick L, Abadi Sh, Einhorn M, Kopelman NM, Salman-Minkov A, Mayzel J, Chay O, Mayrose I. 2015. The Chromosome Counts Database (CCDB) – a community resource of plant chromosome numbers. New Phytol. 206(1):19–26.

Shaked H, Kashkush K, Ozkan H, Feldman M, Levy AA. 2001. Sequence elimination and cytosine methylation

are rapid and reproducible responses of the genome to wide hybridization and allopolyploidy in wheat. Plant Cell. 13:1749–1759.

Skaptsov MV, Smirnov SV, Kutsev MG, Shmakov AI. 2016. Problems of a standardization in plant flow cytometry. Turczaninowia. 19(3):120–122.

Smirnov YA. 1968. Uskorennyi metod issledovaniya somaticheskykh khromosom plodovykh [Accelerated method for studying somatic chromosomes in fruit trees, in Russian]. Tsitologia 10(12):1601–1602.

Song K, Lu P, Tang K, Osborn CT. 1995. Rapid genome change in synthetic polyploids of Brassica and its implications for polyploid evolution. Proc Natl Acad Sci USA. 92:7719–7723.

Stebbins GL. 1971. Chromosomal evolution in higher plants. London: Arnold.

Swanston-Flatt S, Day C, Bailey C. 1990. Traditional plant treatments for diabetes. Studies in normal and streptozotocin diabetic mice. Diabetologia. 33:462–464.

Tang L, Fu L, Lu Ch, Hou X, Shan W, Zhan Zh. 2017. New cytotoxic phloroglucinol derivatives from *Agrimonia pilosa*. Fitoterapia. 118:69–72.

Xiong Z, Gaeta RT, Pires JC. 2011. Homoeologous shuffling and chromosome compensation maintain genome balance in resynthesized allopolyploid *Brassica napus*. Proc Natl Acad Sci USA. 108:7908–7913.

Zenil-Ferguson R, Ponciano JM, Burleigh JG. 2016. Evaluating the role of genome downsizing and size thresholds from genome size distributions in angiosperms. Am J Bot.103 (7):1175–1186.