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INTRASPECIFIC POLYMORPHISM IN WILD AND CULTURED DIPLOID SPECIES OF *MATRICARIA* L. DETECTED BY MOLECULAR CYTOGENETIC MARKERS, ANALYSIS OF MEIOSIS AND ESSENTIAL OIL COMPOSITION

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ABSTRACT

The comparative studies of diploid Matricaria chamomilla L (2n=18) (Azulena and Sybirskaya bisabololnaya varieties), Matricaria discoidea L. (2n=18) and Matricaria inodora L. (2n=18) were carried out by DAPI/C-banding, FISH with 26S and 5S rDNA probes, the analysis of meiosis and composition of essential oil. Based on DAPI/C banding and FISH with 26S and 5S rDNA probes, all the chromosomes in karyotypes of the studied diploid chamomile species were identified, and generalized idiograms of chromosomes with account of all possibilities of DAPI/C-banding patterns as well as chromosomal localization of 26S and 5S rDNA sites were constructed. The results of comparative analysis of DAPI/C-banding and localization of ribosomal genes in the studied related chamomile species conformed to the hypothesis on monophyletic origin of their genomes. In most cases, we observed the classical type of meiosis with formation of 9 **baculiform bivalents. Insignificant percentage of** ***abnormalities suggested genetic stability of genomes of the studied species. The analysis of essential oil composition confirmed that M. discoidea could be used for medical purposes in common with M. chamomilla. The comparative analysis of*** **interspecific karyotype** ***differences and also the qualitative composition of essential oil allowed us to conclude that M^d genome took an intermediate position between M^{ch} and M^i genomes, and M. discoidea was considerably closer to M. chamomilla than M. inodora.***

Keywords C/DAPI-banding, fluorescence in situ hybridization, genome, Matricaria, meiosis.

I. INTRODUCTION

Herbal medicine is considered to be an effective alternative to chemical medicine, and in recent years, scientific interest in the medicinal use of plants has been increased. Today, about forty percent of medicines come from plants or comprise herbal active ingredients. For the last twenty years, the demand for medicinal herbs went up by about twenty-five percent [1].

Wild medicinal plants are used as valuable natural products for obtaining a lot of effective remedies. They are often brought under cultivation for creating sustainable raw materials source. Chamomile (*Matricaria chamomilla* L., syn. *Chamomilla recutita* (L.) Rauschert., *Matricaria recutita* L., 2n = 18) is a well-known ancient medicinal plant species from the aster family (*Compositae* = *Asteraceae*) which is widely used in folk and traditional medicine [2, 3].

The flowers of *M. chamomilla* contain blue essential oil from 0.2 to 1.9% [4, 5]. In the oil, the main component is chamazulene (2.3–10.9%) but there are also such sesquiterpenoids (up to 50 %) as cadinene, farnesene, bisabolol, monoterpenes, myrcene and others [6, 7].

Now a search for producers of biologically active substances as well as donors of useful genes is made within related species of valuable medicinal plants. Wild related species of *M. chamomilla* used in folk medicine [7] include *M. inodora* L. (*Tripleurospermum perforatum* (Merat) M. Lainz =*Matricaria perforata* Merat, *Tripleurospermum inodorum* (L.) Sch.Bip.) (2n = 18) and *M. discoidea* L. (syn.=*M. matricarioides* (Less.) Porter ex Britt.) (2n = 18). The both species possess of some medicinal properties but in a less degree than *M. chamomilla* [8]. In common with *M. chamomilla*, flower heads without flower-bearing stems, gathered early in flowering, are used as drug raw materials. The flower heads contain complexes of valuable biologically active substances but the essential oil compositions in *M. chamomilla*, *M. inodora* and *M. discoideais* are different [9]. In addition, *M. inodora* is gathered for producing insecticides which are safe to use [7].

Reestablishment of natural populations of these valuable species and creation new high yielding varieties by using modern approaches, which include a method of introgressive hybridization, has nowadays become very important owing to strong reduction of wild-growing chamomiles. It is necessary, therefore, to clarify the genomic relationships within chamomile species as well as structural features of their karyotypes, to analyze the process of meiosis in the species and selective forms, and also to determine the content of biologically active substances as they are important main agronomic characters.

The cytogenetic analysis allowed to evaluate chromosome numbers 2n = 18 and 2n = 36 in the species of the genera *Matricaria* [10-16]. The comparative karyotype analysis of cultivars and wild species of chamomile carried out by C-banding allowed us to reveal their varietal and specious particularities [17-20]. Chromosomal analysis of the 36-chromosomal species of chamomile by use of modern techniques confirmed the tetraploid status of wild species *M. inodora* (2n=36) [18, 21]. In addition, nuclear 1C DNA content in some chamomile species was estimated [22, 23].

At the same time, the karyotypes of chamomile species are poorly investigated and they still need special attention. The application of modern molecular cytogenetic techniques for karyotype analysis of the main medicinal diploid chamomile species will make possible to obtain the additional information about structural and functional organization of their genomes and also clarify the taxonomic and evolutionary relationships among different chamomile species. The most appreciable varieties and forms of chamomile will be useful for selection process and creation of new varieties with high content of biologically active substances.

The present paper examines karyotypes of diploid chamomile species *M. chamomilla*, *M. discoidea* and *M. inodora* by DAPI/C-banding, fluorescent *in situ* hybridization (FISH) with rDNA probes, analysis of meiosis and essential oil composition.

II. MATERIALS AND METHODS

2.1. Plant material

The seeds of *M. chamomilla* (Azulena and Sybirskaya bisabololnaya varieties) (2n = 18), *M. inodora* (2n = 18) and *M. discoidea* (2n = 18) were kindly provided by the Botanical Garden of the All Russian Institute of Medicinal and Aromatic Plants of Russian Academy of Agricultural Sciences, Moscow, Russian Federation. The varieties were obtained from wild-growing chamomile populations by mass selection method [24, 25].

2.2. Chromosome spreads

For FISH and DAPI-banding, chamomile root tips were incubated for 16-24 hours at 0℃ in 5 mM solution of DNA intercalator 9-aminoacridine (9-AMA) to inhibit chromosome condensation process and accumulate prometaphase chromosomes [26]. After that, the roots were fixed in 96% ethanol:glacial acetic acid fixative (3:1) and then stored at -20℃. Squashed mitotic chromosome preparations were made according to the technique described earlier with minor modifications [19].

For preparing meiotic chromosomes, young chamomile flower heads at the stage of initiation of white ray flowers were used. Squashed meiotic chromosome preparations were made according to the technique described previously [27]. Chromosome slides were counterstained with 0.125 µg/ml DAPI (4',6-diamidino-2-phenylindole) (Serva, Germany) in Vectashield medium (Vector laboratories, Peterborough, UK).

2.3. Fluorescence in situ hybridization (FISH)

FISH with 26S and 5S rDNA probes (isolated from *L. usitatissimum*) was conducted as described previously [28]. Biotin labelled 26S rDNA probe was detected with FITC-conjugated avidin (Vector laboratories, Peterborough, UK). Digoxigenin labelled 5S rDNA probe was visualized with anti-digoxigenin-rhodamine Fab fragments (Roche Biochemicals, Sussex, UK).

2.4. DAPI-banding

After FISH, chromosome slides were stained with 0.125 µg/ml DAPI (4',6-diamidino-2-phenylindole) (Serva, Heidelberg, Germany) in Vectashield medium (Vector laboratories, Peterborough, UK) to obtain DAPI/C-banding.

2.5. Chromosomal analysis

The slides were examined using Olympus BX61 fluorescence microscope (Olympus, Tokyo, Japan), and images were taken with monochrome CCD camera (Cool Snap, Roper Scientific, USA). The obtained images were processed with Adobe Photoshop 12.1x32 (Adobe, Birmingham, USA). At least fifteen metaphase plates were investigated for each studied chamomile sample. Chromosomes in the karyotypes were identified by analyzing of DAPI-banding patterns with the account of chromosome morphology according to the cytological classification developed early for analyzing of monochrome-, C- and DAPI-banding patterns of *M. chamomilla* and *M. inodora* [17, 18, 21]. The analysis of meiosis was carried out at the stages of metaphase I and anaphase I.

2.6. Determination of essential oil content in the flower heads and chamazulene content in the essential oil

The content of essential oil in the flower heads was determined according to the standard procedure [29]. Chamazulene content in the essential oil was determined according to the State Standard GOST 24027.2-80 “Methods for determination of moisture, ash content, extractive and tannin materials, essential oil” (http://www.gosthelp.ru/gost/gost30604.html) [30].

III. RESULTS

3.1. DAPI/C–banding analysis of karyotypes

The karyotypes of diploid species of *M. chamomilla*, *M. discoidea* and *M. inodora* mainly consisted of metaphase chromosomes ranging from 3 to 5.8 μm. DAPI staining carried out after FISH, which included DNA denaturation and renaturation, revealed C-band-like pattern (DAPI/C–banding) on metaphase chromosomes of the species. (Fig. 1a, c, e).

Similarities in DAPI/C-banding patterns in the karyotypes of all the studied chamomile species were revealed (Fig. 1a, c, e). At the same time, pericentromeric and intercalary bands of the chromosomes in *M. chamomilla* karyotype were larger in size than telomere bands. In *M. inodora* and *M. discoidea* karyotypes, pericentromeric DAPI/C-bands were smaller and telomere bands were larger in size than it was observed in *M. chamomilla* karyotype.

We found that pericentromeric, intercalary and telomere DAPI/C-bands in the karyotypes of all the studied species were rather polymorphic in size. Based on DAPI/C-banding patterns, we identified all the chromosomes in the karyotypes of *M. chamomilla* and *M. inodora* and arranged homologous chromosomes in pairs in *M. discoidea* karyotype according to the cytological classification developed previously [17-19].

In our early studies we had denoted the genome of M. *chamomilla* as M^{ch} genome and the genome of *M. inodora* as M^{i} genome [17, 18]. Accordingly, we denoted the genome of *M. discoidea* as M^{d} genome.

Two pairs of satellite chromosomes (8 and 9) were revealed in karyotypes of *M. chamomilla*, *M. discoidea* and *M. inodora*. The satellites on the chromosomes differed in size and were DAPI-positive. DAPI/C-bands on the satellites of both chromosomes were large in karyotype of *M. chamomilla* (Fig. 1a, c, e) but they were small in *M. inodora*. In karyotype of *M. chamomilla* the DAPI/C-band on the satellite of chromosome 9 was large, whereas it was small on chromosome 8.

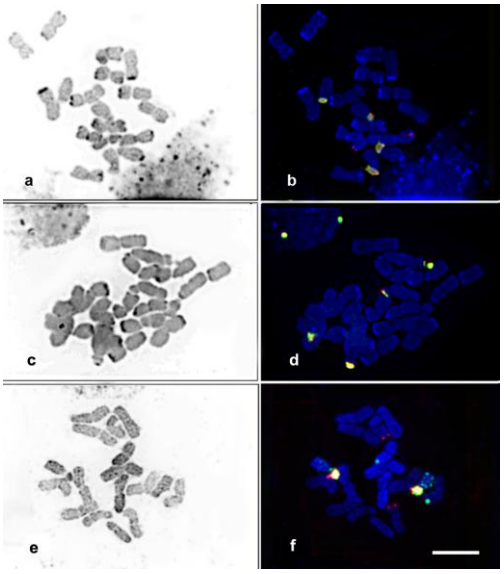

Figure 1 DAPI/C-banding patterns and FISH with 26S (green) and 5S (red) rDNA probes: *M. chamomilla* (2*n* = 2x=18) (a, b); *M. inodora* (2*n* = 2x=18) (c, d); *M. discoidea* (2*n* = 2x=18) (e, f). Bar – 5mkm

3.2. Fluorescence in situ hybridization with 26S and 5S rDNA probes

FISH analysis showed that in the karyotype of *M. chamomilla* co-localized 26S and 5S rDNA sites were revealed in the secondary constriction regions of the satellite chromosomes 8 and 9. Separate 26S rDNA sites were not observed. A separate 5S rDNA signal was detected in the subtelomeric region of the short arm of chromosomes 7 (Fig. 1 b).

In the karyotype of *M. inodora*, we also found co-localized 26S and 5S rDNA sites in the secondary constriction regions of both satellite chromosomes. However, separate 26S or 5S rDNA sites were not revealed (Fig. 1d).

In the karyotype of *M. discoidea*, we observed large intense co-localized 26S and 5S rDNA sites in the secondary constriction region of chromosome 9, but only a separate small 26S rDNA site was revealed on the small satellite of chromosome 8. A separate 5S rDNA signal was detected in the subtelomeric region of the short arm of submetacentric chromosome 7 (Fig. 1f).

Based on chromosome morphology, DAPI-bands localization and also the results of FISH with 26S and 5S rDNA probes, generalized idiograms of chromosomes in M^{ch}, M^{d} and M^{i} genomes of diploid chamomile species *M. chamomilla, M. discoidea* and *M. inodora* with account of all possibilities of DAPI/C-banding patterns as well as 26S and 5S rDNA localization were constructed (Fig. 2).

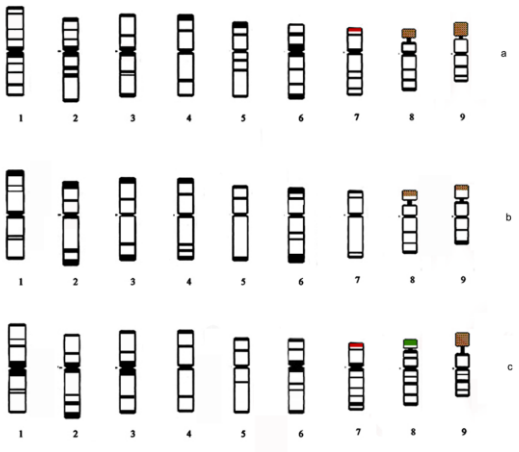

Figure 2 Generalized chromosome idiograms with DAPI/C-banding patterns (black) and localization of 26S (green) and 5S (red) rDNA sites: (a) *M. chamomilla*; (b) *M. inodora*; (c) *M. discoidea*.

3.3. Analysis of meiosis

A cytological study of meiosis was carried out at the stages of metaphase I and anaphase I. In most cases, we observed the classical type of meiosis with formation of 9 baculiform bivalents which mainly moved toward opposite poles of the cell. Chromosome associations (trivalents, quadrivalents) were not detected (Fig. 3). In anaphase I, we observed either cells with normal chromosome disjunction to the poles (9:9) or a few cells (1-2%) with different chromosome aberrations (chromosome lagging, bridges, chromosomal fragments and others). In karyotypes of *M. inodora* and *M. discoidea*, we observed few specific abnormalities (chromosomes agglutinated in a ring at metaphase I or the presence of micronuclei in the tetrads), but the percentage of them was insignificant (1-2%).

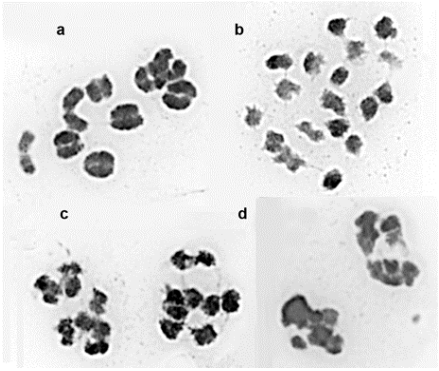

Figure 3 Chromosome associations in meiosis: (a) – *M. chamomilla*, metaphase I; (b) – *M. chamomilla*, prometaphase; (c) – *M. inodora*, anaphase I; (d) – *M. discoidea*, anaphase I and a chromosome fragment.

3.4. Determination of essential oil content in flower heads and chamazulene content in the essential oil

The results of determination of essential oil content in flower heads of the studied chamomile species and also chamazulene content in the essential oil are listed in the table 1. Table 1 shows that the content of essential oil and chamazulene content in the essential oil were higher in *M. chamomilla* (0.72–0.89%; 5.85–8.04%, respectively) than in *M. discoidea* (0.54; 3.7%, respectively). The content of essential oil in *M. inodora* (0.72%) was comparable to that of *M. chamomilla*, but chamazulene was not detected in significant amounts in *M. inodora* (Table 1).

Table 1. The content of essential oil in flower heads and chamazulene content in the essential oil observed in the studied chamomile species

Chamomile species	Moisture, %	Essential oil, %	Chamazulene, %
M. chamomilla, Azulena variety	7.08	0.89	8.04
M. chamomilla, Sybirskaya bisabololnaya variety	6.78	0.72	5.85
M. discoidea	7.12	0.54	3.7
M. inodora	6.98	0.72	-

IV. DISCUSSION

We have previously shown by karyotypic analysis of **C**-banded chromosomes that constitutive heterochromatin of *M. chamomilla* was mainly localized in the pericentromeric chromosome regions. Besides, telomere and intercalary C-bands were also detected [17, 18]. However, only pericentromeric heterochromatin in *M. chamomilla* karyotype was described later [31]. The application of the approach developed previously for analysis of small-sized chromosome plants and based on the use of DNA intercalator 9-AMA, which slowed down the process of chromosome condensation [26], allowed us to obtain longer chromosomes in the spreads (3.0–5.8 µm) and to reveal pericentromeric, telomere and intercalary C-bands on chromosomes of the studied chamomile species [32].

After FISH, chromosome staining with DAPI revealed C-band-like patterns (DAPI/C–banding) (Fig. 1a, c, e) which were rather informative and allowed us to identify every chromosome in the karyotypes of chamomile species according to the cytological classification developed previously for C-banding [17-19]. The mechanisms of C-bandings and DNA-specific fluorochrome staining are known to be different [33, 34]. C-banding reveals constitutive heterochromatin regions (C-bands) whereas DAPI detects AT-rich chromosome regions (DAPI-bands) which are usually localized inside constitutive heterochromatin regions in kyryotypes of many higher plants as DAPI-bands mostly coincide with C-bands [35- 40].

The analysis of DAPI/C-banding patterns showed that heterochromatin in *M. chamomilla* karyotype was mainly located in the pericentromeric and intercalary chromosome regions. In *M. inodora* and *M. discoidea* karyotypes, pericentromeric DAPI/C–bands were smaller and telomere bands were larger in size compared with *M. chamomilla*. In *M. discoidea* karyotype, heterochromatin was distributed more regularly along the chromosomes and the pattern occurred an intermediate position between DAPI/C- banding patterns of *M. chamomilla* and *M. inodora*. In karyotypes of the studied chamomile species, the DAPI/C- bands localized similarly along the chromosomes suggesting that their genomes might have a common origin. There is a strong possibility that differentiation of these genomes was accompanied by redistribution of repetitive DNA and, probably, amplification of its parts. This suggestion was confirmed by nuclear DNA content data. In *M. chamomilla*, DNA content values for 1C nuclei were 2.75-3.87 pcg, whereas in *M. discoidea* and *M. inodora*, it was 2.45 pcg and 2.31 pcg respectively [22, 23]. The results of comparative analysis of DAPI/C-banding patterns in the studied related chamomile species (M^{ch}, M^{i} and M^{d} genomes) conformed to the hypothesis on monophyletic origin of their genomes. Additionally, it was confirmed by the results of chromosomal localization of ribosomal RNA genes obtained by FISH. In every studied chamomile karyotype, we observed two satellite chromosomes and morphologically similar chromosome pairs (Fig. 1b, d, f). However, FISH analysis with 26S and 5S rDNA probes revealed differences in localization of rRNA genes in karyotypes of the species. In *M. chamomilla* and *M. discoidea*, a separate 5S rDNA site was detected in the subtelomeric region of the short arm of chromosome 7. A separate small 26S rDNA site on satellite chromosome 8 was revealed in *M. discoidea*. But separate 5S or 26S rDNA sites were not found in *M. inodora* karyotype. Co-localized 26S and 5S rDNA sites were observed on two satellite chromosomes 8 and 9 in *M. chamomilla* and *M. indora* and on satellite chromosome 8 in *M. discoidea* (Fig. 1b, d, f; Fig. 2).

It is known that 26S and 5S ribosomal RNA genes are arranged as tandem repeats on the chromosomes of most plants. But FISH analysis revealed co-localized 26S and 5S rDNA sites in some *Asteraceae* species, particularly, in *Artemisia*, *Tagetes*, *Helichrysum* and *Tripleurospermum* [41, 42]. Several possible explanations of 26S and 5S rDNA site co-localization were early described. One of them suggested that the co-localization was typical for inferior eukaryotes [43], another one proposed that 5S rRNA genes were occasionally inserted into 26S rDNA [44]. Garcia et al (2010) [42] considered that 26S and 5S rDNA co-localization had occurred during the evolution of plants, especially, the aster family (*Compositae* = *Asteraceae*). Considering that we observed only co-localized 26S and 5S rDNA sites in *M. inodora* karyotype, it might be assumed that *M. inodora* had originated earlier than cultured chamomile (*M. chamomilla* and *M. discoidea*).

The comparative analysis of C- and DAPI-banding patterns of tetraploid *M. inodora* described previously [18, 21] and diploid *M. inodora* showed that the tetraploid karyotype was formed by diploidization of a chromosome set of the same species [18]. The study of meiosis of these two forms confirmed this point of view, as meiotic conjugation of tetraploid *M. inodora* mainly

occurred in common with the conjugation of the diploid form. Furthermore, insignificant percentage of abnormalities could not affect considerably the level of pollen fertility [21].

A cytological study of meiosis in the cells of diploid chamomile forms did not reveal chromosome associations (trivalents, quadrivalents) (Fig. 3). But in anaphase I, we observed cells (1–2%) having different chromosome aberrations: chromosome lagging, bridges, and others, which led to chromosome loss and unequal distribution of genetic material in daughter cells. The insignificant percentage of chromosomal abnormalities in meiosis was not critical in the studied chamomile species, suggesting genetic stability of their genomes. Many such abnormalities were eliminated at the stage of interkinesis (dyads, tetrads), and they had a minor effect on pollen vitality [45]. All the studied chamomile species possessed high quality pollen, as evidenced by a high level of pollen fertility (93–96%).

It is known that quantity and quality of accumulated essential oil depends on climatic and growing conditions [46, 47]. The studied chamomile species were grown in Moscow region, nevertheless, we found differences in the composition of their essential oil in flower heads (Table 1). Table 1 shows that the content of essential oil in *M. chamomilla* was much the same as in *M. inodora* whereas the content of essential oil in *M. discoidea* was less by almost a third. Additionally, chamazulene content in the essential oil of *M. chamomilla* was almost twice the amount of that in *M. discoidea*, and chamazulene in the essential oil of *M. inodora* was not detected in significant amounts. Therefore, the analysis of the composition of essential oil in the studied chamomile species allowed us to conclude that *M. discoidea* was considerably closer to *M. chamomilla* (which is an important medicinal plant) than *M. inodora*. The analysis of qualitative composition of the essential oil of *M. discoidea* showed that it could be used for medical purposes [29].

Thus, the comparative analysis of interspecific karyotype differences and also the qualitative composition of essential oil allowed us to conclude that the M^d genome took an intermediate position between M^{ch} and M^i genomes, and M^d genome could be used as a source of useful (for hybridization) genes during the development of new chamomile varieties with high content of biologically active substances.

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BIBLIOGRAPHY

[1]. Sokolov, S.J. and Zamotaev, I.P. 1988. Textbook of medicinal plants. Moscow, Medical science
[2]. Sokolov, P.D. 1993. Plant Resources of the Soviet Union: family Asteraceae. Nauka, St. Peterburg
[3]. Franz, Ch., Bauer, R., Carle, R., Tedesco, D., Tubaro, A. and Zitterl-Eglseer, K. 2005. Study on the assessments of plants/herbs, plants/herbs extracts and their naturally or synthetically produced components as additives for use in animal production. CFT/EFSA/FEEDAP/2005/01.:155–69.
[4]. Bradley, P.R. 1992. The British herbal compendium. British Herbal Medicine Association, London
[5]. Mann, C. and Staba, E.J. 2002. The chemistry, pharmacology and commercial formulations of chamomile. In: Craker, L.E., Simon, J.E. (ed) Herbs, spices and medicinal plants- recent advances in botany, horticulture and pharmacology Haworth Press Inc, New York. pp. 235–280.
[6]. Konovalova, O.A. and Rybalko, K.S. 1982. Biologically active substances of chamomile. *Rast Resur*, **18:** 116–127.

[7]. Singh, O., Khanam, Z., Misra, N. and Srivastava, M.K. 2011. Chamomile (*Matricaria chamomilla* L.): An overview. *Pharmacogn Rev*, **5:** 82–95.
[8]. Blinova, K.F. and Yakovlev, G.P. 1990. Botanical and pharmacognostic dictionary. High School, Moscow
[9]. Muravjova, D.A., Sam'lina, I.A. and Yakovlev, G.P. 2002. Pharmacognosy. Moscow, Medical science
[10]. Pobedimova, E.G. 1961. *Tripleurospermum* Sch. Bip. in Komarov V. L. *Flora USSR*, **26**: 157-184.
[11]. Koul, M.L.H. 1964a. Chromosome numbers in some medicinal *Composites. Proc Ind Acad Sci*, **59:** 72–77.
[12]. Koul, M.L.H. 1964b. Cytology of some *Composites. J Sci Res Banaras Hindu Univ*, **14**: 20–22.
[13]. Bolkhovskikh, Z.V., Grif, V.G., Zakharyeva, O.I. and Matveeva, T.S. 1969. Chromosome numbers of flowering plants. Nauka, Leningrad, USSR
[14]. Federov, A.A. 1969. Chromosome numbers of flowering plants. Academy of Natural Sciences of the USSR, Leningrad, USSR
[15]. Rostovtseva, T.S. 1979. Chromosome numbers of some species of the family *Asteraceae*. *Bot Zhurn*, **64**: 582-589.
[16]. Abd El-Twab, M.H., Mekawy, A.M. and El-Katatny, M.S. 2008. Karyomorphological studies of some species of *Chrysanthemum sensu lato* in Egypt. *Chrom Bot*, **3**: 41–47.
[17]. Samatadze, T.E., Muravenko, O.V., Klimakhin, G.I. and Zelenin, A.V. 1997. Intraspecific chromosome polymorphism in *Matricaria chamomilla* L. (syn. *M. recutita* L.) studied by C-banding techniques. *Russ J Genet*, **33**: 111–113.
[18]. Samatadze, T.E., Muravenko, O.V. and Zelenin, A.V. 1998. Comparison of C-banded chromosomes in karyotypes of three species of the genus *Matricaria* L. *Russ J Genet*, **34**: 1469–1473.
[19]. Samatadze, T.E., Muravenko, O.V., Popov, K.V. and Zelenin, A.V. 2001. Genome comparison of the *Matricaria chamomilla* L. varieties by the chromosome C- and OR-banding patterns. *Caryologia*, **54**: 299–306.
[20]. Muravenko, O.V., Samatadze, T.E. and Zelenin, A.V. 1998. Computerized and visual analysis of the G-banding-like pattern in chamomile. *Biol Membr*, **15**: 670–678,
[21]. Samatadze, T.E., Amosova, A.V., Suslina, S.N., Zagumennikova, T.N., Melnikova, N.V., Bykov, V.A., Zelenin, A.V. and Muravenko, O.V. 2014. Comparative cytogenetic study of the tetraploid *Matricaria chamomilla* L. and *Matricaria inodora* L. *Biology Bulletin*, **41:** 123–132.
[22]. Garcia, S., Inceer, H., Garnatje, T. and Vallès, J. 2005. Genome size variation in some representatives of the genus *Tripleurospermum. Biol Plant*, **49**: 381–387.
[23]. Kováčik, J., Klejdus, B., Hedbavny, J., Mártonfi, P., Štork, F. and Mártonfiová, L. 2011. Copper uptake, physiology and cytogenetic characteristics in three *Matricaria chamomilla* cultivars. *Water, Air, Soil Pollut*, **218**: 681–691.
[24]. Glasova, M.V. 1978. Selection of chamomile *Matricaria chamomilla* L. in the Moscow region. Dissertation, Main Botanical garden named Moscow the Russian Academy of Sciences
[25]. Glasova, M.V., Perepelova, O.M., Shelud´ko, L.A. and Buzel´, N.A. 1982. Camomilla. Biology, breeding and seed production of the medical cultures. In: Chikov PS (ed) Of Medical plants, Moscow. All Russian Institute of Medicinal and Aromatic Plants, pp 62-68.
[26]. Muravenko, O.V., Amosova, A.V., Samatadze, T.E., Popov, K.V., Poletaev, A.I. and Zelenin, A.V. 2003. 9-aminoacridin- an efficient reagent to improve human and plant chromosome banding patterns and to standardize chromosome image analysis. *Cytometry*, **51**: 52–57.
[27]. Samatadze, T.E., Zelenin, A.V., Suslina, S.N., Amosova, A.V., Popov, K.V., Zagumennikova, T.N., Tsitsilin, A.N., Bykov, V.A. and Muravenko, O.V. 2012. Comparative cytogenetic study of the forms of *Macleaya cordata* (Willd.) R. Br. from different localities. *Russ J Genet*, **48:** 63–69.
[28]. Semenova, O.Yu., Samatadze, T.E., Zelenin, A.V. and Muravenko, O.V. 2006. A comparative study of genomes of flax species of sections *Adenolinum* and *Stellerolinum* using fluorescent hybridization in situ (FISH). *Biol Membr*, **23:** 453–460.
[29]. State Pharmacopoeia of the USSR. 1987. XI (ed), Nauka, Moscow
[30]. GOST 24027.2-80. 1980. Methods for determination of moisture, ash content, extractive and tannin materials, essential oil. IOP Publishing GosthelpWeb. http://www.gosthelp.ru/gost/gost30604.html. Accessed 6 march 1980
[31]. Abd El-Twab, M.H., Mekawy, A.M. and El-Katatny, M.S. 2012. Karyomorphology of six taxa in *Chrysanthemum sensu lato* (Anthemideae) in Egypt and their genetic relationships by Giemsa C-banding. *J Sys Evol*, **50**: 58–63.
[32]. Muravenko, O.V. and Zelenin, A.V. 2009a. Chromosomal organization of the genomes of small chromosome plants. *Russ J Genet*, **45:** 1516–1529.
[33]. Pignone, D., Galasso, I., Rossino, R. and Mezzanotte, R. 1995. Characterization of *Dasypyrum villosum* (L.) candargy chromosomal chromatin by means of in situ restriction endonucleases, fluorochromes, silver staining and C-banding. *Chrom Res*, **3**: 109–114.

[34]. Peterson, D.G., Lapitan, N.L.V. and Stack, S.M. 1999. Localization of single- and low copy sequences on tomato synaptonemal complex spreads using fluorescence in situ hybridization (FISH). *Genetics*, **152**:427–439.
[35]. Scweizer, D. 1980. Fluorescent chromosome banding in plants; applications, mechanisms, and implications for chromosome structure. In: Davids, D.R., Hopwood, D.A. (eds) The plant genome.The John Innes Institute, Norwich, 61–71
[36]. Guerra, M. 2000. Patterns of heterochromatin distribution in plant chromosomes. *Genet Mol Biol*, **23**: 1029–1041.
[37]. Moraes, A.P., Filho, W. and Guerra, M. 2007. Karyotype diversity and the origin of grapefruit. *Chrom res*, **15**: 115–121.
[38]. Muravenko, O.V., Yurkevich, O.Yu., Bolsheva, N.L., Samatadze, T.E., Nosova, I.V., Zelenina, D.A., Volkov, A.A., Popov, K.V. and Zelenin, A.V. 2009b. Comparison of genomes of eight species of sections *Linum* and *Adenolinum* from the genus *Linum* based on chromosome banding, molecular markers and RAPD analysis. *Russ J Genet*, **135**: 245–255.
[39]. Yurkevich, O.Yu., Naumenko-Svetlova, A.A., Bolsheva, N.L., Samatadze, T.E., Rachinskaya, O.A., Kudryavtseva, A.V., Zelenina, D.A., Volkov, A.A., Zelenin, A.V. and Muravenko, O.V. 2013. Investigation of genome polymorphism and seed coat anatomy of species of section *Adenolinum* from the genus *Linum*. *Genet Res Crop Evol*, **60**: 661–676.
[40]. Amosova, A.V., Zemtsova, L.V., Grushetskaya, Z.E., Samatadze, T.E., Mozgova, G.V., Pilyuk, Y.E., Volovik, V.T., Melnikova, N.V., Zelenin, A.V., Lemesh, V.A. and Muravenko, O.V. 2014. Intraspecific chromosomal and genetic polymorphism in *Brassica napus* L. detected by cytogenetic and molecular markers. *J Genet*, **93(1):** 133–143.
[41]. Pellicer, J., Garcia, S., Garnatje, T., Hidalgo, O., Siljak-Yakovlev, S. and Vallès, J. 2008. Molecular cytogenetic characterization of some representatives of the subgenera *Artemisia* and *Absinthium* (genus *Artemisia*, Asteraceae). *Collect Bot*, **27**: 19–27.
[42]. Garcia, S., Panero, J.L., Siroky, J. and Kováčik, J. 2010. Repeated reunions and splits feature the highly dynamic evolution of *5S* and *35S* ribosomal RNA genes (rDNA) in the *Asteraceae* family. *Plant Biol*, **10**: 176–202.
[43]. Srivastava, A.K. and Schlessinger, D. 1991. Structure and organization of ribosomal DNA. *Biochimie*, **73**: 631–638.
[44]. Drouin, G. and Muniz-de-Sá, M. 1995. The concerted evolution of *5S* ribosomal genes linked to the repeat units of other multigene families. *Mol Biol Evol*, **12**: 481–493.
[45]. Sybenga, J. 1996. Recombination and chiasmata: few but intriguing discrepancies. *Genome*, **39**: 473–484.
[46]. Nevruzov, E.N. 1997. Influence Ecological Factors on the Accumulation of Biologically Active Substances in Plants (New and Unconventional Plants and the Perspectives of Their Use), (Proc. 2nd Int. Symposium), **2**: 73–76.
[47]. Fefelova, S.G. 2007. Specificity of Alkaloid and Microelements Accumulation in False Hellebors of East Transbaikal in Relation to Ecological and Phytocenotic Factors. Dissertation, East Siberia State University of Technology and Management

