

CYTOGENETIC RESEARCH REGARDING SPECIES WITH ORNAMENTAL VALUE IDENTIFIED IN THE NORTH-EAST AREA OF ROMANIA

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ABSTRACT - In the paper are presented data regarding the number of somatic chromosome determine at three species of plants with ornamental value from spontaneous flora of Romania (*Allium ursinum* L., *Centaurea phrygia* L., *Dianthus armeria* L.) and cultivated in the experimental field to evaluate the adapt capacity, aiming to use them in crops. For chromosome study was used a root tip meristem obtained from plantlets aged 3-7 days. Chromosome was stained by Feulghen method, and the samples were examined at optic microscope Motic, relevant images being assumed by a video camera and processed with its soft. The obtained results were compared with the ones already existed in the literature, at populations from other ecologic and geographic areas. So at *Allium ursinum*, from Dobrovăț forest (Iași area) all the analysed individuals had a chromosome number $2n=2x=14$, specie being characterized by a great karyological stability. Studies made on *Centaurea phrygia*, from Neamț area, show the presence of tetraploid individuals, with $4n=44$ chromosomes. The specie is known as a diploid one, with chromosome number $2n=2x=22$, but many authors confirm the

existence of diploid and tetraploid populations, separated by geography and ecology. Population of *Dianthus armeria*, identified in Iali area (Bîrnova forest), is a mixt population with $x=15$, formed by tetraploid individuals $4n=60$, but in which could be found also a minor diploid cytotype with chromosome number $2n=30$.

Key words: *Allium ursinum*; *Centaurea Phrygia*; *Dianthus armeria*; Chromosome number.

REZUMAT - Cercetări citogenetice la specii cu valoare ornamentală, identificate în zona de nord-est a României. În lucrare sunt prezentate date cu privire la numărul cromozomilor somatici, determinat la trei specii de plante cu valoare ornamentală, provenite din flora spontană a României (*Allium ursinum* L., *Centaurea phrygia* L., *Dianthus armeria* L.) și cultivate în câmpul experimental pentru evaluarea capacității de adaptare, cu scopul introducerii lor în cultură. Pentru studiul cromozomilor s-a utilizat meristem radicular, obținut din plantule cu vârsta de 3-7 zile. Colorarea cromozomilor s-a realizat după metoda Feulghen, iar

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preparatele au fost examinate la microscopul optic, imaginile relevante fiind preluate cu camera video și prelucrate cu soft-ul corespunzător acestora. Rezultatele obținute au fost comparate cu cele existente în literatura de specialitate, la populații din alte areale ecologice sau geografice. Astfel, la *Allium ursinum*, din pădurea Dobrovăț (Iași), toți indivizii analizați au avut numărul de cromozomi $2n=2x=14$, specia fiind caracterizată printr-o stabilitate cariologică mare. Determinările efectuate la *Centaurea phrygia*, provenită din zona Neamț, au scos în evidență prezența indivizilor tetraploizi, cu $4n=4x=44$ cromozomi. Specia este cunoscută ca diploidă, cu numărul de cromozomi $2n=2x=22$, dar este confirmată, de mai mulți autori, și existența populațiilor diploide și tetraploide, separate atât geografic, cât și ecologic. Populația de *Dianthus armeria*, identificată în zona Iași (pădurea Bîrnova), este mixtă, cu $x=15$, formată din indivizi tetraploizi $4n=60$, dar în cadrul careia se întâlnește și un citotip minoritar diploid, cu număr de cromozomi $2n=30$.

Cuvinte cheie: *Allium ursinum*; *Centaurea phrygi*; *Dianthus armeria*; număr cromozomi.

INTRODUCTION

Chromosome characterization function of number, size, morphologic type helps to establish the karyotype, a basic component for species identification, cytological data being very important in plants' systematic (Stace, 2000, cited by Baltisberger and Widmer, 2009), for motivating some taxonomic decisions, especially in hybrids case. At some species, the basic number of chromosomes didn't change through evolution process but

at others were caused numerous chromosomal mitigations (polyploid) by modification of chromosome number from the ancestor gamet (Sărac, 2005).

Also very important in genetic studies at angiosperms was the diversity of genome size, correlated with different characters at nucleus cellular level or at organism level (estimated time for mitotic and meiotic cell division, reflected in life cycle period; adapt capacity at low temperatures etc.) (Bennett and Leitch, 1997; Ohri and Pistrick, 2001).

Studies regarding chromosome number, ploidy level, DNA content etc. were done at a great number of angiosperm species, inclusively at populations from different areas, being know that the karyological differences are also induced by ecological conditions (Krauhulcová, 2003). There are many angiosperms species at which karyological investigations are missing and at others the results are unsure due to a single population study or even a single individual (Bennett, 1998, cited by Baltisberger and Widmer, 2009).

So, that the informations regarding karyotype of different taxa to be as complete as possible it is recommended that the studies to be done on more individuals from the same population or from populations with different ecological areas and also from zones of confluence areas of related species (Stace, 2000).

The rich floral diversity of Romania offers the possibility of some ample karyological studies on

plants. In literature could be found informations regarding chromosome number of some taxa from different ecologic areas of Romania and which belong to genus *Achillea*, *Podospermum*, *Campanula*, *Dianthus*, *Silene*, *Geranium*, *Nepeta*, *Stachys*, *Teucrium*, *Nigritella*, *Aconitum*, *Consolida*, *Ranunculus*, *Geum*, *Potentilla*, *Veronica*, *Cerastium* etc. (Baltisberger, 2009; Băra *et al.*, 2003; Boşcaiu *et al.*, 1999). A special group is formed by rare and endemic species at which the cytogenetic studies are not so numerous (Băra *et al.*, 2003; Tăcină, 1980).

The continuation of karyological investigations is necessary even at the species with a know basic number of chromosomes to confirm the already existed data. Cytological investigations on species from spontaneous flora with ornamental value to elucidate some aspects regarding the number and morphology of the chromosomes from these species is one of the main aims of the studies undertaken for introduction in crop and their usage as a germplasm source in breeding works, for improving the assortment of ornamental plants.

The paper aims to investigate and to check the existed informations regarding chromosome number at some studied species, the presented data being compared with the one existed in literature.

MATERIALS AND METHODS

Plant material. Karyological studies were made on some plant species identified and gathered from spontaneous flora from North-East of Romania, species considered to have ornamental qualities: *Allium ursinum* L., *Centaurea phrygia* L., *Dianthus armeria* L.

Allium ursinum L. (wild garlic), **Alliaceae** family, geophytes perennial specie (with bulbs) originally from temperate area of Europe, is frequently found, in Romania, in oak and beech forest and present an ornamental interest through small and white flowers, grouped more together (20) in umbel type inflorescences. Flowering take place at the end of spring (may-june). Biological material (bulbs, seeds) was gathered from a population from Iași (Dobrovăț forest).

Centaurea phrygia L. (*C. austriaca* Wild., *C. phrygia* subsp. *phrygia*), **Asteraceae** family, is a hemicytrophite perennial specie, originally from mountain areas of Europe (Oprea, 2005). In Romania is frequently founded on grasslands near the limits of hardwood and coniferous forests (from sessile level up to firtrees). The height is around 1 m and decorates through inflorescences with violet flowers. Biological material (seeds) was gathered from a population from Neamț area.

Dianthus armeria L. (deptford pink) it is an annual specie from **Caryophyllaceae** family, originally from Europe. In Romania is sporadically found on grasslands and sub-shrubs, from steppe area to beech level (Ciocârlan, 2000). Have thin stems, with a heigh of 30-50 cm and decorates by terminal inflorescences, consist of sesil flowers, with pink-red petals spotted with white and accompanied by bracts. Blossom from june till august. Biologic material is

gathered from populations identified in Bîrnova forest (Iași county).

Chromosome counting. For chromosome study was used a root tip meristems obtained from seeds germinated for 3-7 days (moment when the roots reached the length of 1-1.5 cm). Chromosome staining was realized in according with Feulghen method, the samples being examined at optic microscope Motic B1, with zoom 100x, and relevant images were assumed by Motic video camera and processed with its soft.

RESULTS AND DISCUSSIONS

Allium ursinum L. Place: Iași county (Dobrovăț forest)

Genus *Allium* present a great diversity of species distinct both by morphologic features and ecologic habitats and also by cytogenetic characters (basic number of chromosomes, ploidy level and genome type) (Ricroch *et al.*, 2005). The modern ranking accept over 750 species, many of them with recognize a economic importance (Fritsch *et al.*, 2002; Yousaf *et al.*, 2004; Stearn, 1992). In Romania can be found around 30 species from which 26 are wild species (two of them could be found in crops) (Ciocârlan, 2000; Oprea, 2005).

Ecologic, taxonomic, systematic, morphologic, anatomic, karyologic and other studies realised by various authors gave the opportunity to elucidate aspects regarding *Allium* genus.

From kariological point of view, prevail the basic number of chromosomes $x = 7$ and $x=8$

(sometimes also $x=9$), at which are added the polyploid forms. Ploidy level at the species from this genus could be between $2x$ till $16x$ (Bennett *et al.* 2000).

Kawano and Nagai (2005) show that Asian species are reported as tetraploid with $4n=32$, and the ones from Europe are diploid with $2n=16$. At some *Allium* species were identified a variable number of chromosomes (Baranyi *et al.*, 1999; Fritsch *et al.*, 2002).

Allium ursinum is an exception characterised by a great karyological stability. All the data from references present this specie as being diploid with $2n=14$ chromosomes, without recording variation of ploidy level on it (Baranyi *et al.*, 1999; Karpaviciene, 2007).

Our investigations confirm these dates, the determined chromosome number being of $2n=14$ (Fig. 1).

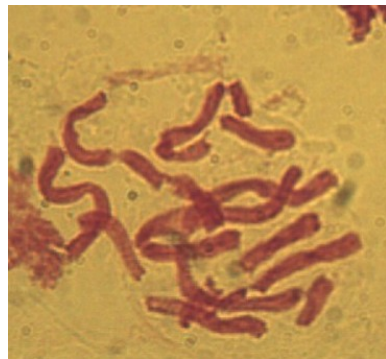


Figure 1 - Somatic metaphase of *Allium ursinum* specie ($2n=14$)

Centaurea phrygia L. Place: Neamț county

In Central Europe (Germany, Czech Republic, Slovakia, Poland,

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Austria, Hungary and West of Ukraine) were identified more of seven taxons of *Centaurea phrygia* group, both species and sub-species (Koutecky, 2007). Dostal (1976), in *Flora Europaea 4* (cited by Koutecky, 2007) identify at *Centaurea phrygia* species a number of nine sub-species. From those ones in Romania were identified six (Oprea, 2005): subsp. *phrygia*, subsp. *melanocalathia* (Borbas) Dostal, subsp. *carpatica* (Porcius) Dostal, subsp. *pseudophrygia* (C.A. Mey.) Gugler, subsp. *ratezatensis* (Prodan) Dostal, subsp. *rarauensis* (Prodan) Dostal.

In literature the basic number of chromosomes at the species of *Centaurea* genus varies from $x = 7$ to $x = 16$ being identified also numerous diploid and tetraploid species.

Centaurea phrygia is a diploid specie with a chromosome number $2n = 2x = 22$ (Dydał et al., 2009). Diploid number of chromosomes was reported at subspecies *Centaurea phrygia* subsp. *pseudophrygia* C.A. Mey., *Centaurea phrygia* subsp. *stenolepis* și *Centaurea phrygia* subsp. *phrygia* (Dostal 1976, Wagenitz 1987 cited by Koutecky P., 2007). In 1989, Dostal identify at *Centaurea phrygia* subsp. *phrygia* diploid forms respectively tetraploids forms (Koutecky P., 2007).

The literature shows a geographical separation of different cytotypes of *Centaurea*, some authors considering only diploid cytotype for *Centaurea phrygia* specie, in Central Europe. In according with some ample studies realised by Koutecky

(2007), diploid populations were recorded in North Bohemia, North Moravia, Eastern Carpathians and only one population in Western Carpathians. Tetraploid populations were identify in Western Carpathians, in Czech Republic and Slovenia by Majovsky et al. (1987) and Dostal (1989), cited by Koutecky (2007).

In conclusion, the existence of diploid and tetraploid populations from this specie is confirmed by many authors, but these cytotypes are separated by geography and ecology.

Data regarding the ploidy level of the population from these species on Romania territory are not clear enough. In “*Flora of Russia: the european part and bordering regions*”, vol VII, by Fedorov and Tzvelev (2003) *Centaurea phrygia*, subsp. *carpatica*, spreaded in western Carpathians on Romania territory, is diploid with $2n=22$ chromosomes.

In our research specie *Centaurea phrygia* is tetraploid with $2n=4x=44$ (Fig. 2).



Figure 2 - Somatic metaphase of *Centaurea phrygia* ($2n=44$)

Dianthus armeria L. Place: Bîrnova forest (Iași county)

Dianthus genus content diploid, tetraploid and hexaploid species, with a number of chromosomes $2n = 2x = 30$, $2n = 4x = 60$ and $2n = 6x = 90$ ($x = 15$). More this genus include diploid, tetraploid and hexaploid cytotypes in more species (Weiss *et al.*, 2002). From 91 species of *Dianthus* genus studied by Carolin (1957) it was ascertain that the majority of species are diploid (67%), 18.7% tetraploid, 6.6% hexaploid and 7.7 % present various cytotypes (Balao *et al.*, 2009).

Chromosome number $2n = 30$ confirm also other previous studies realised on populations from different areas (Fedorov, 1969; Goldblatt and Johnson 1991, 1994, 1998, 2000; Petrova 1975, 1995; van Loon and van Setten, 1982, cited by Pavlova, 2008). Chromosomes of *Dianthus armeria* are small, and the pair of chromosomes have a globular aspect (Pavlova, 2008).



Figure 3 - Somatic metaphase of *Dianthus armeria* $2n=30$

Our studies on *Dianthus armeria* from Bîrnova forest (Iași county) show the existence of some diploid individuals with $2n=30$ chromosomes (Fig. 3) and also of some tetraploid individuals with $4n=60$ chromosomes (Fig. 4), diploid frequency being less.

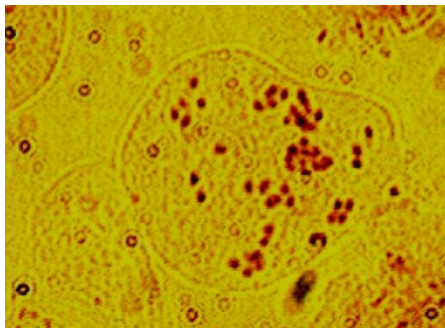


Figure 4 - Somatic metaphase of *Dianthus armeria* $2n=60$

Usually natural populations contain only one cytotype but could be found mixt populations in which together with a preponderent cytotype could appear a minoritar cytotype. After Petit *et al.* (1999) these minority cytotypes appear based on the existance of a previous one asnd could co-habit a period together with the one from wich he derived. In the case *Dianthus* specie genus, at which the phenomena of polyploidy appear frequently, being an important factor of specie, the appearance in a mainly diploid population of some tetraploid cytotypes could be a possible phenomenon. Hybridization of those cytotypes lead to the appearance of some tetraploids individuals, sterils, so the formed cytotypes could co-habit in population for a long period of time being reproductive separated.

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Checking this hypothesis could be done in the future through artificial hybridizations of individuals with different polidy levels, but only the simple appearance of polyploid cytotypes in diploid populations of *Dianthus armeria* suggest the existence of some evolutiv processes

at species' level which could be elucidated by new mollecular studies at this specie.

In *Table 1* are presented synthetically the results of cytogenetic studies realised on *Centaurea phrygia*, *Dianthus armeria*, *Allium ursinum*.

Table 1 - Data regarding the number of mitotic chromosomes in somatic cells of some species from spontaneous flora

Specie	Chromosome number (data from literature)	References	Chromosome (own results)
<i>Allium ursinum</i>	$2n=14$ $x=7$	Baranyi et al, 1999; Karpaviciene, 2007, Ricroch et al, 2005, Bennett <i>et al.</i> 2000	$2n=14$ $x=7$
<i>Centaurea phrygia</i>	$2n=22,44$ $x=11$	Dostal, 1976, 1989; Wagenitz 1987; Majovsky et al. 1987; Pulkina, 1988; Lavrenco et al.; 1990 Hellwig 2004 – cited by Koutecky, 2007 Dydak et al., 2009 Koutecky, 2007	$4n=44$ $x=11$
<i>Dianthus armeria</i>	$2n=30$ $x=15$	Pavlova, 2008	$2n=30$ and $4n=60$ $x=15$

CONCLUSIONS

Allium ursinum is a specie characterized by a great karyological stability, all analyzed individuals having chromosome number $2n=2x=14$.

Centaurea phrygia is a diploid specie with chromosome number $2n = 2x = 22$, but at which is confirmed by many authors the existence of diploid and tetraploid populations separated geographical and ecologic. The studied population, gathered from

Neamț area, is tetraploid, with $4n=44$ chromosomes.

Population of *Dianthus armeria*, identified in Bârnova forest (Iași area), is a mixt population with $x=15$, formed by tetraploid individuals $4n=60$ but in which could be found a minoritar diploid cytotype with $2n=30$ chromosome number.

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