

## PRELIMINARY STUDIES CONCERNING CHROMOSOME CONSTITUTION OF SOME *CORNUS MAS* L. GENOTYPES (BACAU DISTRICT)

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**Abstract:** The cytogenetic characterization is necessary to decipher the controversies on biogeography, taxonomy and evolution of the genus, evolution of chromosome number. Some of *Cornus* species have the following diploid chromosome numbers: *Cornus suecica* L.  $2n=22$ , *Cornus mas* L.  $2n=18, 54$ , *Cornus sanguinea* L.  $2n=22$ , *Cornus sericea* L.  $2n=22$ , *Cornus alba*  $2n=22$ . Our preliminary observations performed on *Cornus mas* genotypes (Bacau district) confirmed the existence of  $2n=18$  chromosomes. Karyotypic formula is  $2n = 18 = 14m + 4sm$ , the centromeres having median and submedian position. Chromosome size ranged between 2.92 – 4.63 microns, with variability limits from 2.86 to 4.71 microns. The length of haploid complement is 33.62 microns. Centromeric index varies from 36.14 to 44.83, while the arm ratio values are comprised in interval 1.23 - 1.77.

### INTRODUCTION

The *Cornaceae* family comprises 15 genera, *Cornus* being the most important from these. This genus includes approximately 65 species of great alimentary and pharmacological value, due to the presence of anthocyanins with antioxidative and anti-inflammatory properties (Seeram et al., 2002), due to edible oil, ascorbic acid (amount double than in oranges, reported to the fresh weight unit), saccharides, pigments, tannins etc. The cornelian cherry seeds are dried and roasted to obtain a powder that is consumed as coffee substitute. The fruits are utilized to prepare soft drinks, jams, jellies, syrups. Also, they can be used as antidiarrheic and in enteritis treatment, while the bark, stem and roots of Cornelian cherry have antifever effect. The wood, very hard and resistant, but homogenous and elastic, is used in turning works or even in construction of some car components. An old legend says the Cornelian cherry plants have been used to build the Trojan Horse. Also, in *Aeneid*, Vergilius noted the utilization of Cornelius cherry wood in spear making. Even the common name of plant comes from its wood hardness (<http://membres.lycos.fr/bouainepa3moine/arbres/arbres.htm>).

This family constituted the object of a big number of studies resorting to modern molecular and cladistic methods in view to evaluate the genetic diversity (Murrell, 1993, Xiang et al., 1996, Caetano-Anolles et al., 1999; Fan and Xiang, 2001, 2003; Xiang et al., 2002; Zhang et al., 2008). The establishment of phylogenetic relationships in *Cornus* genus was possible by methods of molecular genetics allowing the study of DNA profile. For example, the analysis of restriction sites from chloroplast DNA and of some specific regions from DNA (rbcL, matK, 26S rDNA, ITS) led to the elucidation of several controversial aspects concerning the biogeography, taxonomy and genus evolution, number chromosome evolution, morphology and phytochemistry of the representants of *Cornus* genus, and to the characterization and grouping of Cornelian cherry genotypes (Trigiano et al., 2001; Xiang et al., 1996, 2005; Ercisli et al., 2008).

This study is dedicated to the knowledge of diploid chromosome number and of morphological characteristics of mitotic chromosomes in some Romanian provenances of Cornelian cherry, and also to the karyotype construction for the studied genotypes.

### MATERIAL AND METHODS

The seedlings whose root meristematic tips were used for chromosome analysis have been obtained by seed germination of respective genotypes. To obtain maximum volume of data on chromosome number and their morphology, several stages must be carried out. As mitotic inhibitor 8-hydroxyquinoline was used. The slides with metaphase somatic chromosomes were prepared by squash method, and analyzed at Nikon Eclipse 600 microscope, the photos being performed with a Cool Pix Nikon digital camera (100x objective, 1600 x 1200 dpi resolution). The images were processed in Adobe Photoshop program. Our measurements included the total length of chromosomes, length of long arm, length of short arm, arm ratio ( $r = \text{long arm}/\text{short arm}$ ), centromeric index ( $Ci = 100 \times \text{short arm}/\text{total length}$ ), arm difference ( $d = \text{long arm} - \text{short arm}$ ), relative length of each chromosome (expressed as a percentage of the absolute length of each chromosome pair out of the total length of the chromosome complement).

To establish the chromosome morphological type and for the karyotype construction we resorted to Levan's nomenclature (Levan et al., 1964). The chromosome pairs were placed in karyotype in descending order of their size.

## RESULTS AND DISCUSSIONS

Cornelian cherry is considered as a species with medium genetic variability, which now is not subjected to high risks of genetic erosion. Cultivated varieties do not exist, but few selected wild seedlings or clonally propagated are cultivated in yards, fences of farms or as ornamentals in gardens. The *Cornus* species, like other small trees, have a complex and deep dormancy and do not exist infallible method to surpass this state and to promote the germination even the favourable conditions are assured. In nature, the germination of this species becomes in the spring of the second or even third year. The seeds must be very soon separated by fruit pulp, because this contains substances inhibiting the germination. Another factor that makes difficult the germination is the “stony” consistency of the seeds. The results dedicated to the attempts to surpass the dormancy state are more or less satisfactory. Řezníček (2007) used the cold stratification of Cornelian cherry seeds, followed by a stage of 5 intervals of cold-warm alternation and an auxin treatment, but well results were obtained only after two years after seed harvesting. Although we immediately made the separation of seeds from pulp fruits and we tried a large scale of treatments to break the seed dormancy (mechanical scarification, chemical scarification with  $H_2SO_4$ , on variable time intervals, warm and/or cold stratification, GA3 treatments applied for variable periods), the germination percentage was very low.

According to literature, some of *Cornus* species have the following diploid chromosome numbers (STACE, 1997, cf. [www.floranordica.org](http://www.floranordica.org)): *Cornus suecica* L.  $2n=22$ , *Cornus mas* L.  $2n=18$ , 54, *Cornus sanguinea* L.  $2n=22$ , *Cornus sericea* L.  $2n=22$ , *Cornus alba*  $2n=22$ . The DNA content is 6.80 pg/2C (Plant DNA C-values database, Royal Botanic Gardens, Kew).

In P6 – Bălăneasa genotype originated in Bacău district, the metaphases displayed  $2n=18$  chromosomes (fig. 1), number corresponding to that discovered by DUDUKAL, 1984.

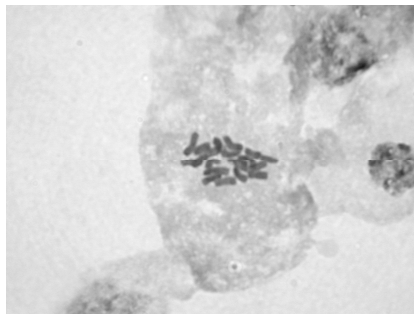


Fig. 1. *Cornus mas* L. – metaphase  $2n=18$

As shown in Table 1, the chromosomes exhibit average lengths varying from 2.92 to 4.63  $\mu m$ , the length of haploid complement being 33.62  $\mu m$ . The values obtained for the average chromosome lengths are very close to those published by Dudukal (1984), respectively 2.9 – 4.6  $\mu m$ . If this author established the existence of 7 metacentric pairs and 2 acrocentric pairs, our measurement allowed us to classify the chromosomes in 7 metacentric pairs, with median placed centromeres, and 2 submetacentric pairs, with chromosomes having submedian placed centromeres. Therefore, the haploid complement of P6 – Bălăneasa genotype have  $7m + 2 sm$  chromosome formula, respectively the karyotypic formula is  $2n = 18 = 14m + 4sm$ .

Table 1. Average values for somatic chromosome features P6 – Bălăneasa genotype of *Corvus mas L.* (Bacău district) (Length of haploid complement = 33.62 microns)

Chromosome pair	Chromosome type	Total length		Long arm		Short arm		Arm ratio	Arm difference (μm)	Centromeric index	Relative length (%)
		μm	Limits of variability	μm	Limits of variability	μm	Limits of variability				
I	m	4.63	4.55-4.71	2.76	2.72-2.81	1.87	1.83-1.91	1.48	0.89	40.38	13.78
II	m	4.45	4.43-4.47	2.53	2.52-2.54	1.92	1.89-1.95	1.32	0.62	43.08	13.23
III	m	3.96	3.92-4.01	2.22	2.20-2.24	1.74	1.71-1.76	1.27	0.48	43.97	11.78
IV	m	3.91	3.89-3.93	2.41	2.40-2.42	1.50	1.48-1.51	1.61	0.91	38.35	11.62
V	sm	3.71	3.69-3.73	2.37	2.37-2.37	1.84	1.33-1.36	1.77	1.03	36.14	11.04
VI	m	3.47	3.43-3.51	2.17	2.16-2.18	1.30	1.27-1.32	1.67	0.87	37.43	10.32
VII	m	3.44	3.41-3.47	1.90	1.88-1.92	1.54	1.54-1.55	1.23	0.36	44.83	10.23
VIII	m	3.13	3.09-3.17	1.97	1.95-1.98	1.17	1.14-1.19	1.23	0.80	37.21	9.32
IX	sm	2.92	2.86-2.92	1.83	1.83-1.87	1.07	1.03-1.11	1.73	0.78	36.61	8.68



Fig. 2. Karyotype for P6 – Bălăneasa genotype of *Corvus mas L.* (Bacău district)

It must also note that other two chromosome pairs – IV and VI – have arm ratios very close to 1.7 (1.61, respectively 1.67); over this limit, the chromosomes could be classified as submetacentric. No secondary constrictions were found, fact also different by the data published by above mentioned author that evidenced satellite like structures. Generally, the karyotypes with small chromosomes (under 4 microns) and preponderantly metacentric and submetacentric are considered to be symmetrical. A symmetrical karyotype is characterized by the predominance of metacentric and submetacentric chromosomes of approximately the same size. Increasing asymmetry can occur either through the shift of centromere position from median/submedian to terminal/subterminal, or through the accumulation of differences in the relative size between the chromosomes of the complement, thus making the karyotype more heterogeneous (Acosta et al., 2005; Paszko, 2006). These symmetrical karyotypes are probably primitive and show relative chromosome stability, being little evolved – they have not been supported significant rearrangements (Stebbins, 1971). The karyotype increased asymmetry suggests the intervention – during species evolution - of some chromosome recombinational and restructuration events.

## CONCLUSIONS

The diploid chromosome number in P6 – Bălăneasa genotype of *Cornus mas* L. (Bacău district) is  $2n=18$ . The average size of chromosomes ranged between 2.2 – 4.63  $\mu\text{m}$ , and the haploid complement length is 33.62  $\mu\text{m}$ . The haploid complement of P6 – Bălăneasa genotype have  $n = 7 m + 2 sm$  chromosome formula, respectively the karyotypic formula is  $2n = 18 = 14 m + 4 sm$ . The karyotype has a high symmetry degree.

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