KARYOTYPE TRAITS IN *ROSA NITIDULA* BESSER

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Keywords: *Rosa nitidula* Besser, karyotype, mixoploidy
Abstract: The knowledge of karyotype characteristics of *Rosa* genus is important because this taxonomic group shows an impressive phenotype, genotype and ecological variability and plasticity. In *Caninae* section, the degree of complexity is amplified by the special chromosome constitution – presence of two genome types (allopolyploid status) and of heterogamous, unbalanced meiosis. Cytogenetic analysis of *Rosa nitidula* Besser genotype evidenced the mixoploidy state, namely the coexistence – in the meristematic apexes of the same individual – of different chromosome numbers (2n=2x=14 and 2n=5x=35). The chromosome size is small (1.22 – 2.50 μm, with limits of variability comprised between 1.21-2.56 μm), and length of haploid chromosome complement is 12.09 μm. The karyotype formula of diploid cells of *R. nitidula* Besser is 2n=2x=14=12m+2sm.

INTRODUCTION

Rosaceae family comprises approximately 100 genera and 1000 species, some of them of great economical importance. According to Kalkman (cf. Dickinson *et al.*, 2007), this family has 85 genera and about 2000 sexual species, but Leus (2005) sustains the existence of 3000 species belonging to the Rosaceae family. At least 32 genera show euploid increases of chromosome number. The complexity of *Rosa* genus, containing about 200 species widely distributed throughout the Northern Hemisphere, is conferred by the impressive phenotype, genotype and ecological variability and plasticity due to some evolutive processes like hybridisation, introgression etc. All these factors are related and interconditionable: hybridisation determined asymmetric meioses, the unequal and asymmetric meiotic distribution of the chromosomes („*canina* meiosis”) leads to gametes with unequal number of chromosomes (egg cell: 2*n*=3*x*, 4*x*, 5*x*=21, 28, 35; pollen grain 2*n*=1*x*=7), and the heterogamy results in the asymmetrical inheritance – mainly matroclinal – of the characters (Wissemann, 2005, 2006). Breeding of *Rosa* species is difficult because of high level of heterozygosity, differences in ploidy levels, particular problems of sexual reproduction and seed germination.

The objectives of this study are the establishment of chromosome diploid number, the analysis of the morphological traits of somatic chromosomes, and the construction of karyotype.

MATERIAL AND METHODS

For cytogenetic analysis, seeds of *Rosa nitidula* (Bistrita 2 genotype) were placed on filter paper moistened with distilled water. The germination was carried out at 22°C, in dark. The roots (10-15 mm in length) were treated with 8-hydroxyquinoline (0.002 mol/L), for 4 h and then fixed in ethanol-acetic acid mixture (3:l) for 24 h at room temperature. The plant material was stored in refrigerator, at 4^0C, in 70 % alcohol. For microscopic observations, the root tips were hydrolyzed in 50 % hydrochloric acid and stained with modified carbol fuchsin solution. The squash preparations were obtained in 45 % glacial acetic acid and they have been analyzed at Novex Holland K-range microscope with 20x objective; the cells with well-spread metaphase chromosomes were then photographed at Nikon Eclipse 600 microscope with digital camera Cool Pix Nikon, 1600x1200 dpi, 100x objective. The images were processed by Adobe Photoshop programmer.

Chromosome measurements included *length of individual chromosomes* (C), *long arm length* (L), *short arm length* (S), *arm ratio*, r (r = L/S), *centromeric index*, CI (CI=S/C x 100), and *the relative length of each chromosome, %* (C/length of haploid complement x 100). Karyotype was performed according to Levan *et al*. (1964) nomenclature. The chromosome homology and the establishment of the chromosome types were assigned on the basis of centromere position, respectively on CI and r values: the chromosomes are metacentric (r<1.70, CI=37.5-50.0), submetacentric (r=1.70–2.99, CI=37.5–25.0), subtelocentric (r= 3.00–6.99, CI=25.0–12.5), and telocentric (r=7.00–∞, CI<12.5). In karyotypes, the chromosome pairs have been ordered in decreasing size.

RESULTS AND DISCUSSIONS

The karyotypes, which describe the complement phenotypes in terms of number, size, arm ratio, and other chromosome specific features, are dynamic structures evolving through

numerical and structural changes. The above mentioned parameters may differ even between closely related taxa.

Rosa seeds often take two years to germinate. They are characterized by a complex and strong state of dormancy and do not exist efficient and infallible methods to quicken the germination, even if all favourable conditions are ensured. The factors making difficult the seed germination is the hard woody pericarp and the presence of some chemical inhibitors. To surpass the double dormancy, the cold stratification, sometimes accompanied by chemical scarification (with concentrated H_2SO_4) or alternation of seed exposure to high and low temperatures or treatment with gibberellic acid are recommended. Some studies on dog-roses evidenced a superior seed germination efficiency after thermic treatment (12 weeks at 20^0C, followed by 12 weeks at 5^0C), comparatively with exclusively cold exposure (24 weeks, 5^0C). Despite of large palette of the treatments applied to optimize the germination efficiency, the results are not satisfactory.

In *Caninae* section, the degree of complexity is amplified by the special chromosome constitution – presence of two genome types (allopolyploid status) and of heterogamous, unbalanced meiosis (Uggla, 2004, De Cock, 2008). The principal methods used to analyse the ploidy status, reproduction mode and genetic constitution of the plants resulted from interspecific crosses are flow cytometry and molecular markers (RAPD, microsatellites).

Two groups naturally exist in *Rosaceae* family: one (subf. *Rosoideae*) with x=7, 8, 9, another (subf. *Pomoideae*) having x=17. Approximately 200 species are included in *Rosa* genus; for 147 of them the chromosome number was established (Crane and Byrne 2003, cf Roberts *et al.*, 2008). The *Rosa* genus exhibits a typical polyploid series with a basic chromosome number of 7 (from 2n=2*x*=14 to 2n=8*x*=56). The chromosome numbers are multiple of basic number x=7. Aneuploidy is rare (Kroon and Zeilinga, 1974).

The chromosome variability in family and genus is very large. Diploid species (2n = 2x = 14) are *R. arvensis, R. sempervirens* and *R. rugosa*, whereas *R. gallica, R. glauca, R. pendulina, R. pimpinellifolia, R. villosa* are tetraploid (2n = 4x = 28). *R. canina*, *R. agrestis, R. elliptica, R. micrantha, R. montana, R. rubiginosa, R. sicula, R. tomentosa* have pentaploid constitution (2n = 5x = 35). *R. pouzinii* and *R. trachyphylla* are hexaploid species (2n = 6x = 42), in this group some authors including also *Rosa agrestis*. As octoploid species (2n = 8x = 56) is reported *R. acicularis*. Even intraspecific variation was evidenced in *R. sherardii*, *R. mollis* and *R. micrantha* in which shrubs showing ploidy degrees of 4x, 5x and 6x have been described. Polyploidy has long been recognized as an important force in evolutionary diversification and is one of the most important cytogenetic mechanisms in plant evolution and rapid speciation (Stebbins, 1971; Grant, 1981; Levin, 2002, cf. Jian *et al*., 2011).

In *R. canina* x *R. pendulina* „Everest" hybrid series, out of plants with expected 49 somatic chromosomes, a plant with 77 chromosomes was identified. It seems that this increased number is due to chromosome doubling during development of embryo sac at a moment when reduction has already taken place (Zeilinga, 1969).

Except *Caninae* section, all rose species have in most cases 2x or 4x, with a normal Mendelian meiosis. Generally, the species of *Caninae* section are pentaploid, although tetraploid (2n = 4x = 28) and hexaploid species (2n = 6x = 42) have been found (Henker, 2000, cited by De Cock, 2008). Organisms with *canina* meiosis are named hemisexual (Nybom *et al*., 2004).

Fig. 1. Metaphase and karyotype in *Rosa nitidula* (Eistrita 2 genotype)

Table 1. Metric characteristics of mitotic chromosomes in *Rosa nitidula* (Bistrita 2 genotype)

Chromosome pair	Chromosome type	Total length		Long arm		Short arm		Arm ratio	Arm difference	Centromeric index	Relative length
		µm	range	µm	range	µm	range				
I	m	2.50	2.43-2.56	1.36	1.32-1.40	1.14	1.11-1.14	1.20	0.19	45.59	20.65
II	m	2.24	2.21-2.26	1.28	1.26-1.29	0.96	0.95-0.97	1.33	0.30	42.92	18.49
III	m	1.75	1.74-1.75	1.02	1.01-1.04	0.72	0.73-0.72	1.42	0.32	41.29	14.44
IV	m	1.66	1.62-1.70	0.96	0.93-0.98	0.70	0.69-0.72	1.36	0.23	42.32	13.74
V	m	1.38	1.36-1.40	0.74	0.72-0.75	0.64	0.63-0.65	1.15	0.11	46.55	11.41
VI	sm	1.35	1.34-1.37	0.86	0.86-0.87	0.49	0.48-0.50	1.75	0.38	36.31	11.19
VII	m	1.22	1.21-1.23	0.72	0.70-0.74	0.50	0.50-0.51	1.43	0.20	41.10	10.11
Length of haploid complement		12.09									

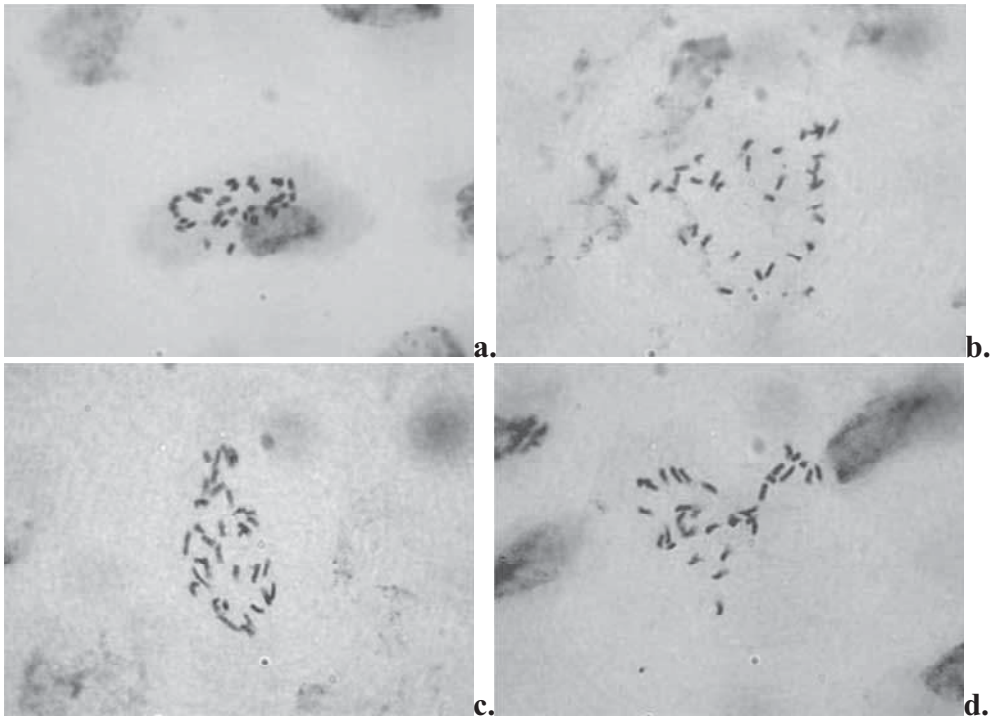

Fig. 2. *Rosa nitidula* – pentaploid (2n=5x=35) metaphase (**a**) and prometaphases (**b**, **c**, **d**)

This kind of meiosis determines unequal, asymmetric distribution of chromosome in gametes (2n = 1x = 7 for pollen grain, 2n = 3x, 4x, 5x = 21, 28, 35 for megaspore), fact resulting in matroclinal inheritance of the characters: 4/5 of nuclear genome is provided by maternal parent, only 1/5 coming from paternal parent (Ritz and Wissemann, 2003). Although this system is complicated, the dog-roses maintain a mixed mating type, ranging from facultative apomixis, selfpollination, geitonogamy (the pollination of a flower stigma with the pollen from other flower on the same plant), to outbreeding reproduction (Wissemann *et al*., 2007).

In *Rosa* genus, the studies concerning chromosome number, karyotype and frequency of meiotic configurations are numerous (Liu and Li, 1985; Subramanian, 1987; Ma and Chen, 1991, 1992; Ma *et al.,* 1997, cf. Fernández-Romero *et al*., 2001), but no analysis of chromosome characteristics of *R. nitidula* Besser we found. Based on arm ratio and chromosome length values, the authors established that in *Rosa* genus the karyotypes are symmetric and that genomic uniformity exists in this genus.

In *Rosa nitidula* genotypes, the primary cytogenetic analysis evidenced the mixoploidy state, namely the existence – in the meristematic apexes of the same individual – of different chromosome numbers. The metaphases presented in Fig. 1 and 2 belong to the same individual and confirm the coexistence in the same tissue of the cells with different ploidy levels: diploid (2n=2x=14) and pentaploid (2n=5x=35). Different ploidy levels (4x, 5x, 6x) show also other *Rosa* species such as *R. sherardii*, *R. mollis*, *R. micrantha*. It seems that the mixoploidy, namely the coexistence of different ploidy levels in the same individual, is more often present in genera

having small chromosomes and contributes to the increase of adaptive potential of respective plants, especially in unfavourable environment conditions.

As Table 1 shows, the size of chromosomes in *R. nitidula* ranges from 1.22 μm to 2.50 μm, with limits of variability comprised between 1.21-2.56 μm; the length of haploid chromosome complement is 12.09 μm. As other authors reported, rose chromosomes are small and difficult to observe through cytology (Leus, 2005). The chromosomes showed a relative length ranging from 10.11 μm (pair VII) to 20.65 μm (pair I). The average values of arm ratio and centromeric index allowed that except one pair (VI), which have submedian placed centromere (sm), the others (pairs I-V and VII) are metacentric chromosomes with median placed centromere (m).

The karyotype formula of diploid cells of *R. nitidula* Besser is 2n=2x=14=12m+2sm. Because of small chromosome size and of the difficulty in obtainment of sufficient metaphases with satisfactory chromosome contraction and spreading, in this moment we can not made remarks about the real nature of the ploidy in the case of 5x state and thoroughgoing studies are necessary to clear this problem. For example, for *R. praelucens* Byhouwer decaploid (2n=10x=70) it was established to be an allopolyploid, respectively an interspecific hybrid (interspecific hybridization is common in *Rosa* genus). In this polyploid tree, like in *R. nitidula*, according to Lima-de-Faria (1980) classification, the chromosomes are also small (under 4 μm, respectively (1.77 – 3.78 μm).

The small size and the morphostructural characteristics, as well as the presence of chromosomes only of m and sm type are the principal arguments to sustain that the karyotype of *Rosa nitidula* Besser is relatively little evolved, with a high degree of symmetry.

CONCLUSIONS

In analyzed *Rosa nitidula Besser* genotype, the mixoploidy was evidenced, namely the coexistence of diploid (2n=2x=14) and pentaploid (2n=5x=35) cells.
Chromosomes are small (1.22 – 2.50 μm).
Only one chromosome pair has submedian placed centromere (sm), the other six have metacentric chromosomes with median placed centromere (m).
The karyotype formula of diploid set is 2n=2x=14=12m+2sm; the karyotype has a high degree of symmetry.

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Acknowledgments. The research was supported by project 52-142/15.09.2008 from PNCD II national research program (2009-2011)