CITOLOGICAL ASPECTS OF THE *ONOBRYCHIS* GENUS

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Abstract. We have examined five species of the *Onobrychis* variety: *O. viciifolia*, *O. crista-galli*, *O. caput-galli*, *O. montana* and *O. transcaucazica*. For each one of these species, we have established the karyotype and determined the chromosome number, length, ploidy level, as well as some features of the pollen. We have identified both diploid and tetraploid species. The basic chromosomal number for the *O. crista-galli* species is eight, namely seven for the *O. viciifolia*, *O. caput-galli*, *O. montana* and *O. transcaucazica* species. Related to the length of the chromosomes, the largest dimensions are encountered at the *O. viciifolia*, while the smallest at *O. transcaucazica*.

INTRODUCTION

Sainfoin (*Onobrychis viciifolia*) is a leguminous perennial herb of the *Fabaceae* family, indigenous in Southern Europe and in temperate regions of Western Asia. Sainfoin has been widely cultivated in Europe as a forage crop. There are two main types of sainfoin, the common type and the giant type. The common type cultivars (or *bifera* type) grow very slow in the establishment year. Common types of sainfoin will out persist by several years. The giant type is far more vigorous in the first year, and will provide a measurable yield during the season, but will only persist for a relatively short period of time. Sainfoin thrives on calcareous soils, with a pH of 6.0+, too dry or too barren for clover and alfalfa. There is also some evidence that suggests that sainfoin will grow well on soils that are low in phosphorus.

The forage legume sainfoin is an important crop well adapted to the dry and semi-arid regions, where the cultivation of alfalfa is restricted by the environmental conditions. Sainfopin grows very well in calcareous chalky soils as well as in the soils with high water. Sainfoin improves the nitrogen content of the soil by fixing the atmospheric nitrogen. Sainfoin is also a very palatable forage plant and since it does not induce bloat, strip grazing of green forage is possible.

This plant is sometimes associated with the Christmas story of the Infant Jesus. Sainfoin is also called esparcet and holy clover. It has many positive characteristics as a forage legume. In ruminants the positive characteristics tend to be the results of the high level of condensed tannins (Krall and Delaney, 1982). Condensed tannins bond to the protein complex in the sainfoin; this protects the protein complex from hydrolyzing in the rumen. This ability to prevent hydrolysis in the rumen allows the protein complex to pass into the abomasums where it is digested (Van Soest, 1994). Furthermore, animals grazing on sainfoin are much less prone to bloat than when grazing other legumes.
Agronomically, it’s positive characteristics include a deep taproot that allows the plant to be very resistant to drought and, since it is a legume, there is a high level of a residual fertility after a sainfoin ley has been ploughed.

Sainfoin presents an amazing variability, sometimes intraindividual, caused even by the climatic and soil conditions. There are intermediary forms of transition from one variety to another. Because of this reason, there are problems regarding the botanical limits of the *Eubrychis* section. The botanical classification of the species may be simplified if there were cytological differences between the genotypes analysed.

**MATERIALS AND METHODS**

Five species of the *Onobrychis* genus were the object of a cytotaxonomical study designed to detect relations between these species and perhaps some evolutionary mechanisms within the genus. The species used were: *Onobrychis crista-galli, Onobrychis caput-galli* of the section *Lophobrychis* and *Onobrychis viciifolia, Onobrychis montana* and *Onobrychis transcaucazica* of the section *Eubrychis*.

In order to determine the number of somatic chromosomes, we used meristematic tissue of the embryonary roots, from seeds recently germinated. The same biological material was also used to determine the chromosome morphology and dimensions. After the harvest, the embryonary roots were previously treated with an alfa-brominenaphtalena solution for four hours in order to block out the cellular division in the metaphase stage and also to establish the contraction of chromosomes. The fastening was made with an ethanol-acetic acid solution (3:1), for three hours. The colouring was made using the Feulgen method, with a Schiff reactive. For the hydrolysis we used hydrochloric acid 1N at 60°C for eight minutes. The metaphases were studied with an optical microscope type Motic equipped with a photographic camera. In order to study the biometrical characteristics of pollen we used mature anthers, coloured in acetic carmine (0.5%). We marked with an x the basic chromosomal number, namely with an n, respectively 2n the haploid and the diploid number of chromosomes.

Pollen material was obtained from field populations and from collections of the Cluj-Napoca University of Agricultural Sciences and Veterinary Medicine. The pollen was acetolysed in the standard way (Erdtman, 1966). For light microscopy, slides were prepared by mounting the pollen in glycerol jelly. Twenty measurements of each feature were made at a magnification of x 1200. Three features were measured: polar diameter, equatorial diameter and polar diameter-equatorial diameter ratio.

**RESULTS AND DISCUSSIONS**

The determinations made upon the chromosome number and dimensions are displayed in table number 1.

*Onobrychis viciifolia Scop.* is a tetraploid species: 2n=28; x=27. Favarger (1954) noticed the presence of two types of SAT chromosomes, belonging to the origin genomes, which compound the karyotype of this species. The phenotypical expression of the SAT chromosomes is variable and it requires the analysis of a large number of metaphases in order to establish the difference between the two types.

The morphological effects of polyploidy within this species are out striking. They become visible especially at the level of the reproductive organs, mostly at fruits and seeds.
The chromosomes number less influence the vegetative organs; they react particularly to the gene constitution.

Average length of the longest chromosome: 3.79µ;
Average length of the shortest chromosome: 1.6µ;
Average length of chromosomes: 3.39µ.

*Onobrychis caput-galli* is a diploid species: 2n=14; x=7. Eskilsson (1963) signalised the existence of intraspecific polyploidy within this species, caused by the geographical conditions and he mentioned triploid forms (2n=21). As far as it concerns our analyses, all 52 plants studied were diploid. This species presents a pair of submetacentric SAT chromosomes.

Average length of the longest chromosome: 2.32µ;
Average length of the shortest chromosome: 1.24µ;
Average length of chromosomes: 1.63µ.

*Onobrychis crista-galli* is a diploid species: 2n=16; x=8. This species also presents a pair of submetacentric SAT chromosomes. Species with the basic chromosome number eight possess a karyotype that is more asymmetric and more specialized than that of the species with the basic chromosome number seven.

Average length of the longest chromosome: 2.23µ;
Average length of the shortest chromosome: 1.08µ;
Average length of chromosomes: 1.58µ.

The variation of the chromosome number at the analysed species

<table>
<thead>
<tr>
<th>Species</th>
<th>Basic chromosomal number (x)</th>
<th>Haploid chromosome number (n)</th>
<th>Somatic chromosome number (2n)</th>
<th>Average length of chromosomes</th>
<th>Variation limits of chromosome length</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. vicifolia</em></td>
<td>7</td>
<td>14</td>
<td>28</td>
<td>3.39</td>
<td>2.74-1.62</td>
</tr>
<tr>
<td><em>O. crista-galli</em></td>
<td>8</td>
<td>8</td>
<td>16</td>
<td>1.58</td>
<td>2.23-1.08</td>
</tr>
<tr>
<td><em>O. caput-galli</em></td>
<td>7</td>
<td>7</td>
<td>14</td>
<td>1.63</td>
<td>2.32-1.24</td>
</tr>
<tr>
<td><em>O. montana</em></td>
<td>7</td>
<td>7</td>
<td>14</td>
<td>1.46</td>
<td>1.86-1.12</td>
</tr>
<tr>
<td><em>O. transcaucazica</em></td>
<td>7</td>
<td>14</td>
<td>28</td>
<td>1.27</td>
<td>1.57-0.69</td>
</tr>
</tbody>
</table>
Table 2. The characteristics of the *Onobrychis* genus flower

<table>
<thead>
<tr>
<th>Species</th>
<th>Corolla (mm)</th>
<th>Calix (mm)</th>
<th>Standard (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. viciifolia</em></td>
<td>9-12</td>
<td>5.5-7</td>
<td>9-12</td>
</tr>
<tr>
<td><em>O. crista-galli</em> (2x)</td>
<td>8-10</td>
<td>5-7</td>
<td>6.5-8</td>
</tr>
<tr>
<td><em>O. crista-galli</em> (4x)</td>
<td>9-11</td>
<td>7-8.5</td>
<td>8-10</td>
</tr>
</tbody>
</table>

The measurements of three features of the studied taxa are displayed in table 3. In equatorial view, the pollen grains are elongated, elliptic to rectangular, and in polar view they are circular or trainagular.

Table 3. Taxa examined for pollen types, with measurements (µm) of the mean of the ranges for the polar and equatorial axes and the shape index (polar:equatorial)

<table>
<thead>
<tr>
<th>Species</th>
<th>Polar axes (µm)</th>
<th>Equatorial axes (µm)</th>
<th>Polar:equatorial ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. viciifolia</em></td>
<td>30.2 – 38.9</td>
<td>15.8 – 20.2</td>
<td>1.91 – 1.96</td>
</tr>
<tr>
<td><em>O. caput-galli</em></td>
<td>31.4 – 42.8</td>
<td>18.7 – 24.6</td>
<td>1.69 – 1.73</td>
</tr>
<tr>
<td><em>O. montana</em></td>
<td>32.6 – 40.6</td>
<td>15.2 – 18.4</td>
<td>2.14 – 2.20</td>
</tr>
<tr>
<td><em>O. crista-galli</em> (2x)</td>
<td>25.4 – 36.0</td>
<td>13.6 – 18.3</td>
<td>1.86 – 1.97</td>
</tr>
<tr>
<td><em>O. crista-galli</em> (4x)</td>
<td>37.2 – 43.2</td>
<td>18.4 – 19.2</td>
<td>2.02 – 2.25</td>
</tr>
</tbody>
</table>

CONCLUSIONS

1. Species with the basic chromosome number eight (*Onobrychis crista-galli*) possess a karyotype which is more asymmetric and more specialized than that of the species with the basic chromosome number seven.

2. *Onobrychis caput-galli* is the connecting link between two sections of the genus, because cytotaxonomically it is more close to the section of *Eubrychis* than the other species of its own section.

3. Chromosome fragmentation is an evolutionary mechanism through the increase of the basic evolutionary process of the genus, at least in some side branches of the genus.

4. The cytological deviations of individuals of *Onobrychis viciifolia* are partly consequences of a karyotype which is little stable.

5. The fodder plant sainfoin (*Onobrychis viciifolia*) has a natural election mechanism in favour of the normal plant; this means that plants with constant numbers of the normal chromosome complement and display cytological regularity.

6. With respect to its polyploidy nature *Onobrychis viciifolia* seems to be of a more recent origin.
REFERENCES