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# StUDY ON THE USE OF FLOW CYTOMETRY IN *PRIMULA VULGARIS*

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## ABSTRACT

*In this study, the use of flow cytometry was investigated for determining and estimating the nuclear DNA content in Primula vulgaris, an ornamental species and important model in genetics and biodiversity studies.The biological material was represented by samples of young leaves harvested from spontaneous populations of deciduous forests in Hunedoara County. Cytometric analysis was performed using a flow cytometer, and the data obtained were processed to evaluate the quantitative variations of nuclear DNA.The results revealed a clear distribution of nuclear populations corresponding to different phases of the cell cycle and allowed the identification of possible ploidy variations between samples. The method proved efficient for the rapid characterization of plant material, providing reproducible and relevant results for genetic and breeding studies. In conclusion, flow cytometry constitutes a valuable tool in the genomic analysis of the species Primula vulgaris, facilitating the assessment of genetic variability and contributing to conservation and selection programs.*

## INTRODUCTION

The species *Primula vulgaris*, belonging to the *Primulaceae* family, represents an important biological model in studies of plant genetics, ecology and evolutionary biology. It is widely distributed in temperate regions of Europe, being frequently found in deciduous forests, wet meadows and hilly areas, where it contributes to the maintenance of biodiversity and ecosystem stability (Barrett, 2019; Rankou et al., 2015). In Romania, *Primula vulgaris* is well represented in hill and submontane forest ecosystems, including deciduous forests in the west of the country. The area located between the localities of Dobra and Bătrâna (Hunedoara County) is characterized by hilly landscapes belonging to the Poiana Ruscă Mountains, where beech (*Fagus sylvatica*), hornbeam (*Carpinus betulus*) and oak (*Quercus spp*.) forests predominate. These habitats offer optimal development conditions for *Primula vulgaris*, due to moist, well-drained soils and moderate shade. This area also has a high biodiversity and a relatively low degree of anthropization in certain areas, which makes it suitable for studies on the genetic variability of natural populations (Petcov, 2025). In this context, sampling biological material from

deciduous forests located between Dobra and Bătrâna offers the opportunity to investigate relatively stable and well-adapted populations to local conditions. Their analysis can provide relevant information regarding the genetic structure, adaptability and response of the species to the environmental conditions specific to the area. In modern plant biology studies, cytogenetic and molecular methods play an essential role in characterizing genetic variability. Among them, flow cytometry has emerged as a rapid and accurate technique for determining nuclear DNA content and ploidy level. It allows the analysis of a large number of cells in a short time, providing essential quantitative data for genetic and evolutionary studies (Doležel et al., 2007; Loureiro et al., 2023). The application of flow cytometry in the study of Primula vulgaris is particularly relevant due to its genetic complexity, including the presence of heterostyly, a mechanism controlled by a supergene involved in outcrossing (Li et al., 2018). Determining variations in DNA content and identifying possible ploidy differences between the analyzed populations can contribute to a better understanding of the adaptation of the species to local ecological conditions, such as those in the deciduous forests of Hunedoara County. The use of flow cytometry also has important implications for biodiversity conservation, allowing the assessment of genetic diversity and the identification of vulnerable populations in the context of climate change and anthropogenic pressures (Pellicer et al., 2021; Forrest & Miller-Rushing, 2010). The integration of cytometric data with ecological information specific to the study area contributes to the development of an interdisciplinary perspective on plant population dynamics.

Therefore, the present study, based on the analysis of *Primula vulgaris* populations from deciduous forests located between Dobra and Bătrâna, aims to highlight the usefulness of flow cytometry in characterizing genomic variability and in understanding the adaptation mechanisms of this species in specific natural conditions.

## MATERIAL AND METHODS

The biological material used in the study was represented by individuals belonging to the *Primula vulgaris* species, taken from the spontaneous flora. The harvesting was carried out from the deciduous forests located in the Dobra – Bătrâna area (Hunedoara County), an area belonging to the geographical unit of the Poiana Ruscă Mountains. The harvesting habitat is characterized by beech (*Fagus sylvatica*), hornbeam (*Carpinus betulus*) and oak (*Quercus* spp.) forests, with moist, well-drained soils and semi-shady exposure, conditions favorable to the development of this species. The samples were collected during the vegetation period (spring), when the plants show intense metabolic activity.

Healthy plants, without signs of stress or degradation, were selected for analysis. Young leaf fragments were taken from each individual, considered optimal for determining DNA content due to the high rate of cell division. The samples were transported under cooling conditions and processed as soon as possible after harvesting to prevent degradation of the biological material. The analysis of nuclear DNA content was performed using the flow cytometry technique, a method widely used in plant cytogenetics studies to determine genome size (Doležel et al., 2007).
Sample preparation: Plant tissue fragments (approximately 0.5–1 $cm^2$) were finely minced with a blade in a specific extraction buffer to release the nuclei. The resulting suspension was filtered through a filter (30–50 µm) to remove cellular debris.DNA staining: Isolated nuclei were stained with a DNA-specific fluorochrome, such as propidium iodide (PI), in the presence of RNase to eliminate RNA interference. The

fluorescence intensity is proportional to the amount of nuclear DNA.Cytometric analysis: The samples were analyzed using a flow cytometer, which allows the measurement of the fluorescence emitted by each nucleus. For each sample, thousands of nuclei were recorded, obtaining characteristic histograms of the distribution of DNA content. The data obtained were interpreted by comparing the position of the fluorescence peaks.



Fig.1 The biological material used *Primula vulgaris*-original

## RESULTS AND DISCUSSIONS

Cytometric analysis was performed on biological material belonging to the *Primula vulgaris* species, taken from the spontaneous flora of the Dobra – Bătrâna area. The evaluation of the samples by flow cytometry allowed the determination of the distribution of nuclei according to the DNA content and the assessment of the quality of the nuclear suspension. The fluorescence histogram (FL1) highlights the presence of a well-defined main peak, corresponding to the G0/G1 phase, i.e. it is in the presynthesis or postsynthesis phase of the cell cycle, located around the value of ~100 relative fluorescence units. (Fig. 2 ) This peak represents the majority population of nuclei and reflects the diploid (2C) DNA content, characteristic of somatic cells in a resting state or in the initial phase of the cell cycle. The coefficient of variation (CV) associated with this peak is approximately 6–7 %, indicating a moderate dispersion of fluorescence values. Although it does not reach the optimal level (<5%), (Table 1) this range is considered acceptable for samples from plant material collected from the natural environment. Dot plot analysis (SSC vs. FL1) confirms the presence of a compact, well-demarcated population of nuclei, indicating efficient isolation of nuclear material. (Fig. 2) The results obtained confirm that the flow cytometry method is suitable for the analysis of DNA content in *Primula vulgaris*, even in the case of samples from spontaneous flora. The presence of a well-defined G0/G1 peak indicates good integrity of the nuclei and proper sample preparation, which is essential for the accuracy of the determinations (Doležel et al., 2007). Such CV values are frequently encountered in studies on spontaneous plants and are considered acceptable in an ecological context (Loureiro et al., 2023). The absence of a well-defined G2/M peak may indicate reduced mitotic activity at harvest, which is typical for perennial plants at certain phenological stages. Also, the lack of additional peaks suggests the absence of detectable polyploidy or aneuploidy phenomena, which indicates genomic stability of the analyzed population. The results are consistent with previous studies on the genus *Primula*, which highlight a stable genomic organization, despite the genetic complexity associated with heterostyly (Li et al., 2018). At the same time, cytometric analysis provides a valuable tool for complementing genetic and ecological data, contributing to the understanding of the

species' adaptability in specific habitats, such as the deciduous forests of the Dobra – Bătrâna area.

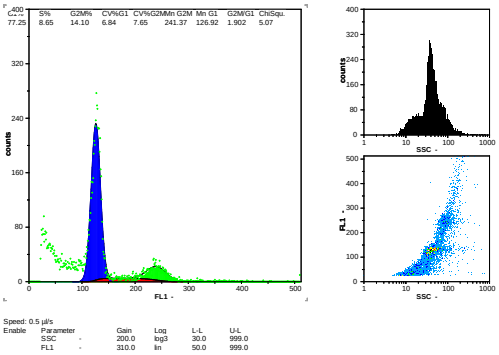



Fig.2 Distribution of nuclear DNA content in *Primula vulgaris* determined by flow cytometry

## CONCLUSIONS

Cell cycle analysis by flow cytometry using propidium iodide reveals a typical distribution for a predominantly stable cell population, characterized by a majority proportion of cells in the G0/G1 phase (77.25 %). The moderate fraction of cells in the S phase (8.65 %) confirms the existence of DNA replication activity. The percentage of cells in G2/M (14.10 %) falls within limits compatible with a normal progression of the cell cycle, without abnormal accumulations indicating mitotic blocks. The quality parameters of the analysis support the robustness of the results: the reduced coefficients of variation and the G2/M:G1 ratio close to the theoretical value (~2) confirm the accuracy of the quantification of the DNA content, and the Chi-square value indicates an adequate fit of the analysis model.

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