

## Report on the outcomes of a Short-Term Scientific Mission<sup>1</sup>

Action number: CA18201

Grantee name: Dhimiter Peci

### **Details of the STSM**

Title: **The application of flow cytometry to plant conservation**

Start and end date: 18/03/2024 to 22/03/2024

### **Description of the work carried out during the STSM**

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

*(max. 500 words)*

The main goal of this STSM was to gain expertise in applying flow cytometric analyses to study plant cytogenetic, investigate ploidy variability on plant species and explore the role of cytogenetic diversity for plant conservation. The Short-Term Scientific Mission was connected to the WG 1 task 1.2 Identification of the potential uses of population biology, conservation genetics in conservation action and it is carried out in the Centre for Functional Ecology, Department of Life Science, University of Coimbra, Portugal.

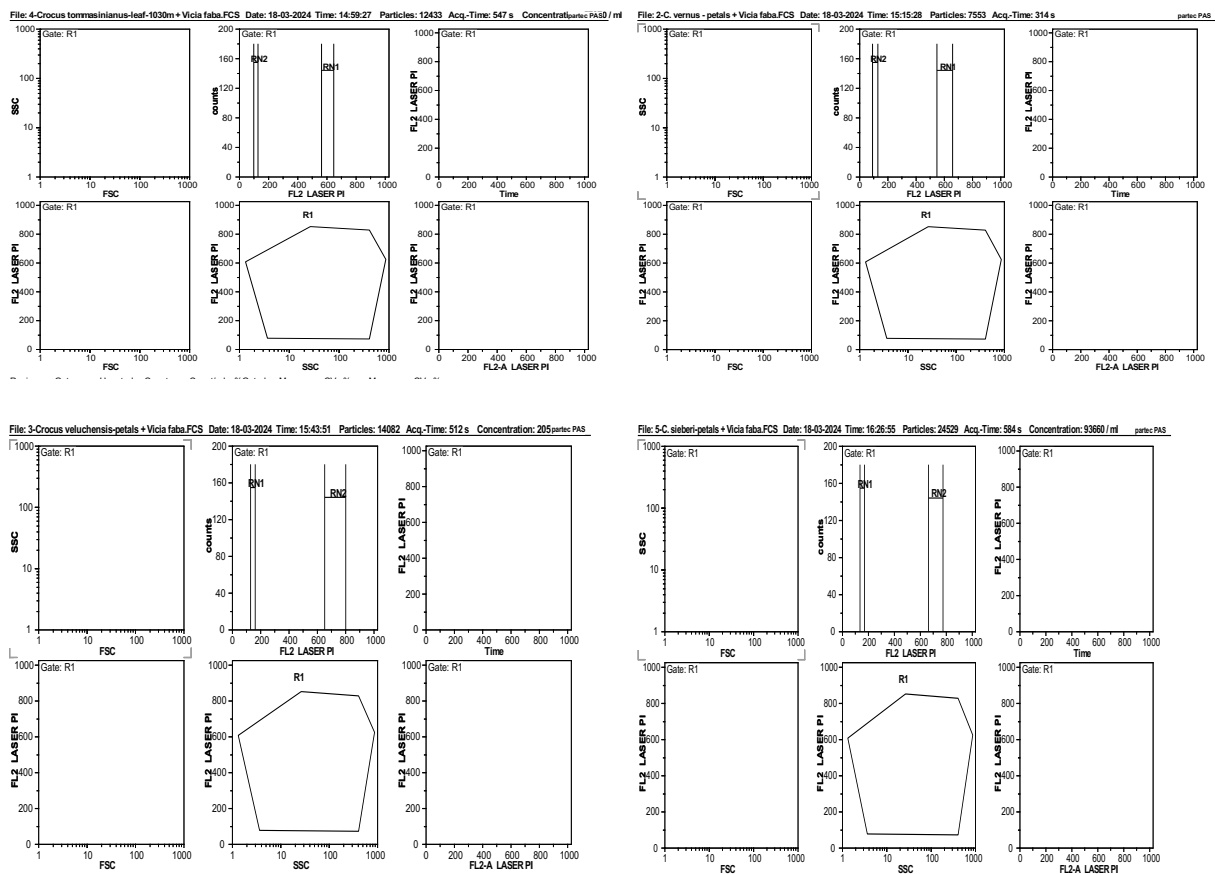
Flow cytometry has emerged as a powerful tool in plant conservation, enabling the estimation of ploidy levels and cell's nucleus size and genome. Ploidy is linked with the ability of a plant to adapt and resist abiotic factors. Thus, polyploid plant species can adapt to a wide range of environmental conditions by developing stress tolerance. Knowledge of the ploidy of a species would assist in their conservation effectively. Additionally, ploidy and genome size estimation serves as a valuable tool for taxonomic classification in cases of uncertainty. Therefore, by acquiring proficiency in these techniques is important and results provided by flow cytometer technique and formulate conservation strategies tailored to the needs of different plant species. As a case study to achieve the STSM objective the flow cytometric analysis was carried out in several *Crocus* species (Iridaceae), which comprise a diverse group of flowering ornamental and medicinal plants distributed in a wide range of habitats. They show considerable variation in morphology, flower colour, size and shape and in ecological adaptation. Polyploidy estimation plays a crucial role in species adaption. Therefore, characterizing ploidy and

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<sup>1</sup> This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

genome size variation provides valuable insights into the adaptive potential of these species and their conservation status.

Plant material of *Crocus* sp. (*C. tommasinianus*, *C. vernus*, *C. veluchensis*, *C. sieberi*) was collected in different altitudes (1000-1220m a.s.l) in the mountain area in Albania. Nuclear DNA was assessed by flow cytometry. Measurements were taken by extracting nuclei from two types of plant material: fresh leaves and petals. To estimate the genome size the internal standard of *Vicia faba* was used. Leaf tissues of the sample and internal standard were co-chopped, and the resulting nuclei suspension was stained with PI buffer, and the measurements were performed in a flow cytometer (Sysmex CyFlow Space). The relative fluorescence intensity  $\geq 1500$  of stained nuclei was performed and the positions of G0/G1 peaks were recorded. The data analysis was carried out by means of data analysis the FlowMax software. The genome size was estimated in pg. The training involved practical laboratory work, including the isolation of nuclei from the plant tissues, fluorescent dye staining procedure, analysing stained nuclei for estimation of genome size and ploidy level, and regarding software and instrumental settings. The training also included a session on data analysis and interpretation.



**Figure 1.** The measurement of *Crocus tommasinianus*, *Crocus vernus*, *Crocus Veluchensis*, *Crocus sieberi* genome size and ploidy against *V. faba* standard

As a result of the analysis *Crocus* sp (as a case study) resulted that they were diploid and the genome size varied from 4.89 to 5.78 pg.

### **Description of the STSM main achievements and planned follow-up activities**

This STSM enabled training on the new techniques to estimate the ploidy and genome sizes in plant species. The acquired knowledge would enable the establishment of new methodology and to benefit

significantly from results provided by flow cytometer technique and formulate conservation strategies tailored to the needs of different plant species in Research Centre of Flora and Fauna, University of Tirana, Albania (home institution). In addition, the STSM laid the foundation for a future collaboration with the team of the host institution for future collaborative projects on the conservation of rare plant species. In particular, the proposed research made a valuable contribution to the action objectives, aimed to identify best practices and share the knowledge on tools and techniques used in plant conservation, as well as to the promotion of the collaboration between institutions involved in this action.