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Analysis of genetic variability in *Vitis vinifera* L. using flow cytometry

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Abstract:

The species *Vitis vinifera* L represents one of the most important cultivated plants worldwide, having a high economic value in the wine industry and a particular relevance in scientific research. The genetic characterization of this species is essential for understanding the variability, genomic stability and mechanisms of adaptation to environmental conditions. In this context, the genetic analysis of the *Vitis vinifera* L. species is highlighted by using flow cytometry, a modern and efficient method for determining the nuclear DNA content and evaluating the level of somatic cell division. The biological material analyzed was represented by plant samples harvested from grapevine crops, subjected to the specific methodology for obtaining nuclear suspensions. The results obtained revealed distributions corresponding to the phases of the cell cycle, indicating a stable genome and the absence of significant ploidy variations. Thus, the application of flow cytometry in the study of the species *Vitis vinifera* L. offers valuable insights into the organization and stability of its genome.

• Introduction

The species *Vitis vinifera* L. represents one of the most important horticultural plants cultivated worldwide, having an essential role in the viticulture and wine industry, as well as a significant contribution to the global agricultural economy. The high genetic diversity of this species, reflected in the large number of existing varieties and clones, is the result of a long process of domestication, selection and vegetative propagation. This genetic variability has direct implications on the adaptability to environmental conditions, productivity and quality of grapes. The integration of flow cytometry with modern genomic analysis technologies contributes to a deeper understanding of genome organization and function, providing relevant information on cellular dynamics and genetic variability.

• Material and method

- The biological material used in the study was represented by young leaves belonging to the species *Vitis vinifera* L., harvested from plants grown in *ex situ* conditions in farms maintained without treatments and highly resistant to diseases that produce black grapes with a "strawberry" aroma.(Fig 1) The leaves were selected in an active physiological stage, without symptoms of diseases or pest attacks, to ensure the accuracy of subsequent analyses. The samples were collected under controlled conditions and transported to the laboratory in sterile containers, at low temperature, to prevent degradation of the cellular material.
- For the determination of the nuclear DNA content, the flow cytometry method was used, according to the protocol. Small fragments of leaf tissue were taken from each plant sample and mechanically minced in a specific extraction buffer to obtain a suspension of nuclei. The suspension obtained was filtered through a fine sieve (30–50 μm) to remove cellular debris and impurities. (Fig.1) Subsequently, the nuclei were stained with a DNA-specific fluorochrome, such as propidium iodide (PI), in the presence of an RNase-type enzyme to eliminate RNA, thus avoiding interferences in the determination of DNA content. The samples were incubated for a short period of time, in the dark, to stabilize the DNA-dye complex. The analysis of the samples was performed using a flow cytometer, which allowed the measurement of the fluorescence emitted by the stained nuclei and, implicitly, the determination of the relative nuclear DNA content. The data obtained were represented in the form of histograms, highlighting the distribution of nuclei in the different phases of the cell cycle (G0/G1, S and G2/M)



Fig. 1 Biological material used *Vitis vinifera* L.-original

• Results and discussions

Flow cytometric analysis of the nuclear DNA content of *Vitis vinifera* revealed the presence of two distinct nuclear populations, corresponding to the main phases of the cell cycle. The fluorescence histogram (FL1) indicated a first peak (Peak 1) with an average intensity value of approximately 69.4 arbitrary units, representing 17.56% of the total events analyzed, and a second peak (Peak 2), with an average of approximately 325.8 units and a weight of 82.44%. (Fig.2)

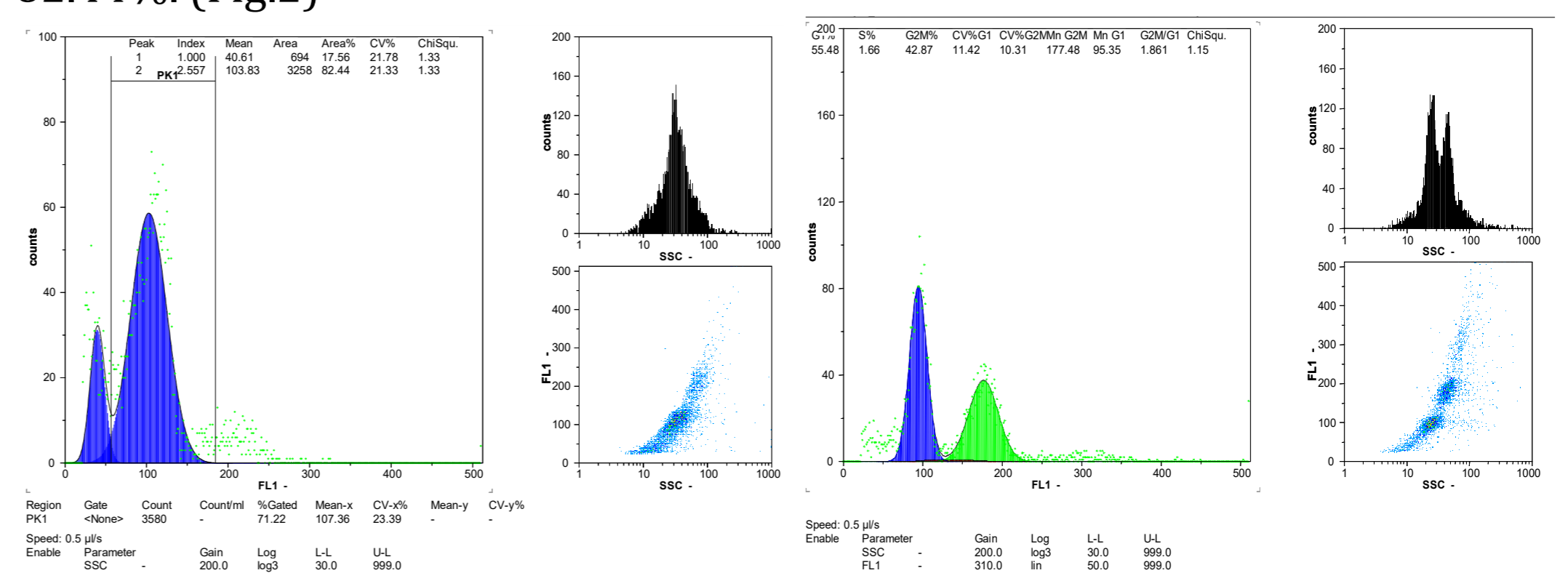


Fig. 2 Histogram of cell cycle phase distribution (G0/G1, S, G2/M) in *Vitis vinifera* L.- initial determination and optimized determination

These results indicate a relatively balanced distribution between G0/G1 and G2/M phases, with a reduced fraction in the synthesis (S) phase. The ratio between G0/G1 and G2/M phases ($G2/G1 \approx 1.88$) is higher than that commonly reported for fully differentiated plant tissues, suggesting a significant accumulation of nuclei in the post-replicative stage. (Fig.3) In both cases, the presence of two main peaks corresponding to the 2C and 4C levels confirms the absence of major ploidy changes, indicating the genomic stability of the analyzed plant material. (Fig.2) (Table 1)

Table 1 Comparison of flow cytometric parameters of DNA content in *Vitis vinifera* L

Parameter	Initial determination	Optimized determination
Phase G0/G1 (%)	17,56	55,48
Phase S (%)	Nedelimitată clar	1,68
Phase G2/M (%)	82,44	42,87
Report G2/G1	~4,69	~0,77
Peak position G0/G1 (Mean)	~69,4	~96,35
Peak position G2/M (Mean)	~325,8	~177,48
Index G2/G1	~2,55	~1,88
CV G0/G1 (%)	21,78	11,42
CV G2/M (%)	21,33	10,31
Histogram resolution	Low	Moderate-good
Cell cycle distribution	Unhinged	Relatively balanced
Data quality	Low	Improvement

• Conclusions

Flow cytometric analysis of DNA content in "old" *Vitis vinifera* highlights the following main aspects: Genomic stability: Both determinations confirm the presence of a stable diploid genome, without indications of aneuploidy or polyploidy. Variability of cell cycle distribution: The significant differences between the two analyses suggest the influence of technical and physiological factors on the distribution of nuclei in the phases of the cell cycle. Practical relevance: Flow cytometry proves to be a useful method for the rapid assessment of genomic stability in traditional plant material, but the interpretation of the data must be carried out in correlation with the experimental conditions