Flow cytometric analysis of Ploidy level

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Agenda

- Materials and methods
- Tomato ploidy assay (DAPI staining)
- Tomato ploidy assay (PI staining)
- Conclusions

Materials and methods

- ACEA NovoCyte 3000 (488, 642, 405 nm lasers), 13 colors
- Competitor cytometer 1 (DAPI staining)
- Competitor cytometer 2 (PI staining)
- Reagents and samples, provided by Prof. Jaroslav Doležel, Institute of Experimental Botany

Flow cytometric analysis of Ploidy level

- Because the nuclear DNA content of G1 nucleus reflects the ploidy of a cell, estimation of DNA content is frequently used for ploidy determination.

Ploidy	DNA Content (G1phase)
n	1C
2n	2C
4n	4C

Table 1. Relation between the ploidy and DNA content of G1 phase nuclei

Flow cytometric analysis of Ploidy level

- Flow cytometric analysis involves the estimation of DNA content and not microscopic evaluation of chromosome number. Thus, the terms Ploidy and DNA ploidy should be used to distinguish between karyotype and DNA content analysis, respectively.

- Main advantages of flow cytometric assay are:

- **Rapid, precise and convenient** (several hundred samples per working day)
- **No need for pure single cell suspension preparation**
- **Non-destructive** (requires small amount of tissue)
- **Analysis of large populations of cells** (detection of subpopulations - mixoploidy)

DNA ploidy analysis using external standard

- The instrument is calibrated using nuclei isolated from a plant with known ploidy, e.g. 2n (the position of the G1 peak is recorded). All other samples are characterized by the relative position of their G1 peaks. Units are thus "C-values"

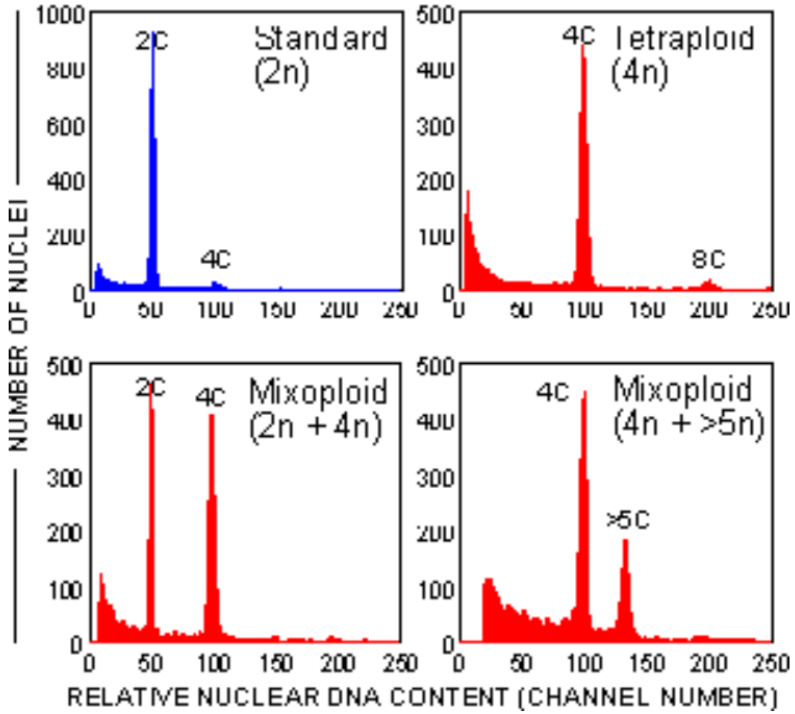

Figure 1. Histograms of relative nuclear DNA content of nuclei isolated from young leaves of Cassava plants (untreated control and plants regenerated from in vitro culture after treatment with a polyploidizing agent)

DNA ploidy analysis using internal standard

- The nuclei of the standard with known ploidy and the nuclei of the sample are isolated, stained and analyzed simultaneously. The DNA ploidy of the sample is then estimated using the ratio of G1 peaks (units are "C-values").

- **Internal standardization eliminates the risk of error** due to variations in sample preparation and instrument instability. It is recommended for precise DNA ploidy estimation (especially when aneuploidy is suspected).

Sample preparation

1. Chop a small amount of plant material (typically 20 mg) with a new razor blade or a sharp scalpel in 0.5 ml of ice-cold Otto I buffer in a petri dish.

2. Add 1 ml of Otto II buffer. It is preferable to include DAPI (or propidium iodide + RNase) in the Otto II buffer. Alternatively, these compounds can be added to the sample after the addition of Otto II buffer. The stains are used at the following concentrations: DAPI, 4 µg/ml; propidium iodide, 50 µg/ml + RNase, 50 µg/ml.

3. Mix well with a pipette.

Sample preparation

4. Filter the suspension through a 50 µm nylon mesh.

5. Store at room temperature, analyzing within 5 - 15 min.

6. Analyse relative DNA content of isolated nuclei.

Browning due to phenolic compounds may be inhibited by adding 2µl/ml ß-mercaptoethanol to Otto II buffer prior its use. This procedure gives good results only with some species. If the results are not satisfactory, it is recommended to test a standard two-step procedure.).

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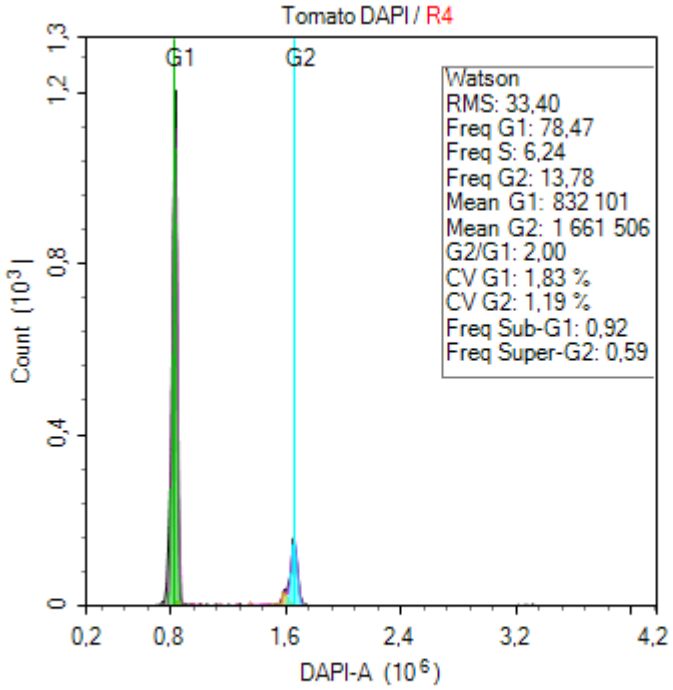
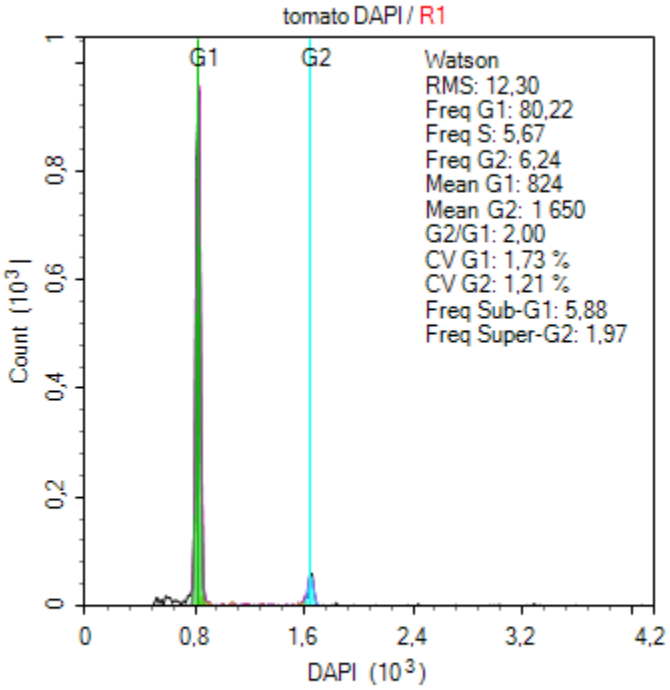
Tomato- DAPI

NovoCyte



RMS	Freq G1	Freq S	Freq G2	Mean G1	Mean G2	G2/G1	CV G1
33,40	78,47	6,24	13,78	832 101	1 661 506	2,00	1,83 %

Competitor 1



RMS	Freq G1	Freq S	Freq G2	Mean G1	Mean G2	G2/G1	CV G1
12,30	80,22	5,67	6,24	824	1 650	2,00	1,73 %

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- **Tomato ploidy assay (PI staining)**
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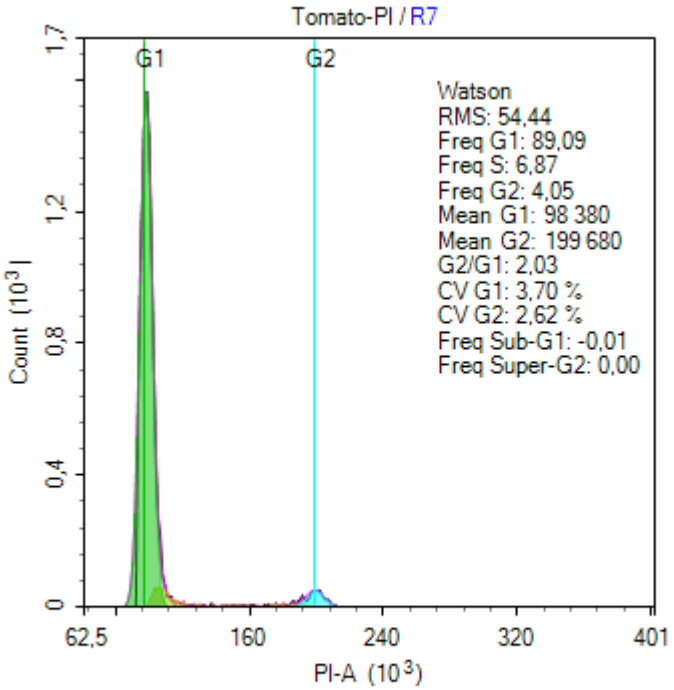
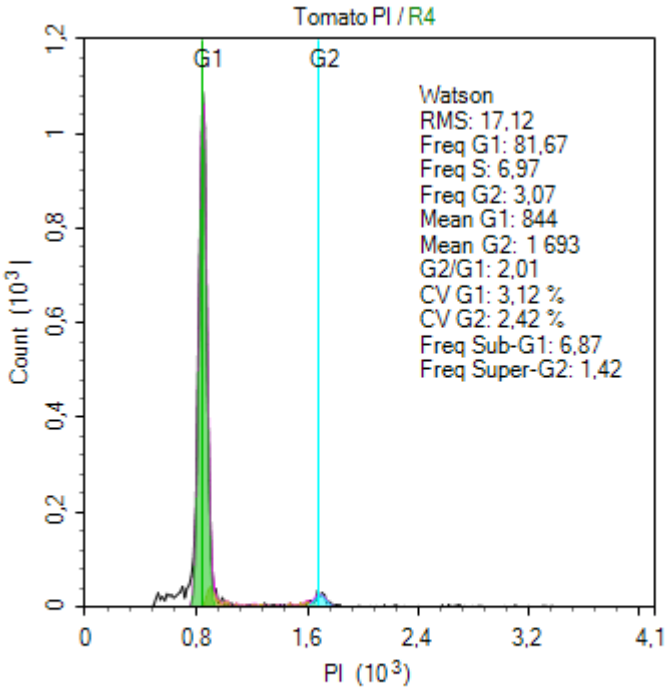
Tomato- PI

NovoCyte



RMS	Freq G1	Freq S	Freq G2	Mean G1	Mean G2	G2/G1	CV G1
54,44	89,09	6,87	4,05	98 380	199 680	2,03	3,70 %

Competitor 2



RMS	Freq G1	Freq S	Freq G2	Mean G1	Mean G2	G2/G1	CV G1
17,12	81,67	6,97	3,07	844	1 693	2,01	3,12 %

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Conclusions

- **Globally, both systems showed comparable CV's**

- **<u>NovoCyte did not require PMT's adjustment</u>, the device is able to read and save the whole data. Competitor cytometers required optics alignment and PMT's gain adjustment prior acquisition.**

- **The obtained results have proved high sensitivity and flexibility of NovoCyte flow cytometer, as a system suitable for accurate and reproducible plant DNA assays.**

References

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- *DAPI staining of fixed cells for high-resolution flow cytometry of nuclear DNA*. In: Crissman HA, Darzynkiewicz Z (eds.). Methods in Cell Biology. Vol. 33., Pp. 105 - 110. Academic Press, New York, 1990**.**
- **http://olomouc.ueb.cas.cz/book/dna-flow-cytometry**

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