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AN EFFICENT AND SOLID METHOD FOR A HIGH YIELD EXTRACTION OF NUCLEI FROM MICRO AMOUNT OF FRESH PLANT TISSUE

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Purpose

Flow cytometry is at the moment one of the most reliable analysis for plant ploidy level, genome size estimations and cell cycle analysis (**Galbraith et al. 2021**). Plant sample preparation is the key step for a successful analysis; the procedure itself it's easy but is: a little labour intensive and time consuming. We have developed a new improved sample preparation technique which with the use of a semi-automatic sampling and robust analytical capabilities of the Cytoflex S instrument, allows to obtain an highly efficient, fast and reliable sample analysis using smaller amount of plant material, reducing the disposables, solutions and time.

Methods

Plant material:

Leaf tissues and roots were obtained from several plant species grown both *in vitro* and *in vivo*.

Sample preparation (Fig1):

Single root tips or leaf pieces of about 1mg (fresh weight) were collected and placed into a single well of a 96 multi well plate (flat bottom) previously filled with 100µL of a buffer solution, selected between LB01 (**Dolezel et al. 1989**), LB05 and TrisMgCl$_2$ (**Pfosser et al. 1995**); DAPI and PI+RNAse were used as DNA fluorescent stains (**Loureiro et al. 2021**). Plant samples were homogenized at 7000 rpm for 13 seconds inside the wells by using an MiniTurrax homogenizer with GN5 probe. At the end of the extraction process, 100µL of the buffer solution were added to reach a final volume of 200µL per well. FCM analysis were performed with Cytoflex S (Beckman Coulter, USA) equipped with 4 lasers and a semi-automatic 96 wells plate loader.

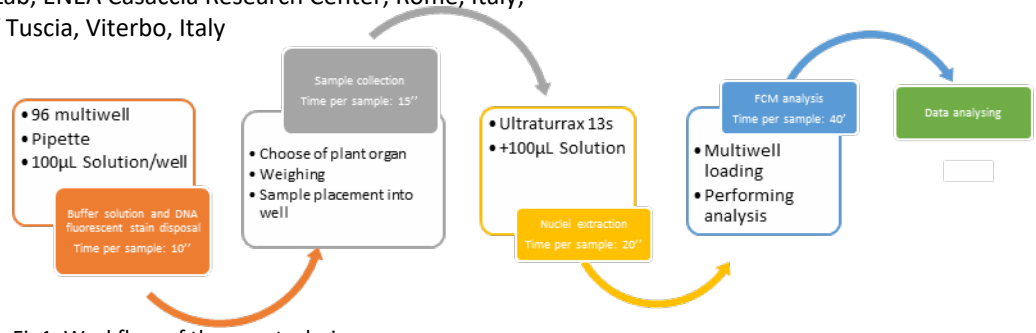

Fig1. Workflow of the new technique

Results

In figure 2, we report the nuclei suspensions obtained, and the relative FCM DNA ploidy evaluation histograms. In table 1, we summarize the results of the extraction procedure obtained from roots and leaves of three plant species (*Lepidium sativum, Triticum durum* cv Simeto, *Solanum Lycopersicon* Moneymaker). FCM Analysis were realized collecting at least 5000 events (nuclei) in 90 seconds per sample at 10µl/min speed. Our technique performed best when smaller amounts of plant tissues were used; increasing the quantity of the plant material (ie. Triticum durum cv Simeto) we did not report any increase in quality and/or quantity of isolated nuclei. For most of the tested species, the CV of G1 nuclei anlyzed spanned from 4% to 7%, which are acceptable values considering how fast and frugale is our procedure. Cytoflex S semi automatic sampling procedure was very effective in FCM analysis, resulting in a 3hours run for a 96 samples. Non a single clogging event occurred while running our nuclei suspensions.

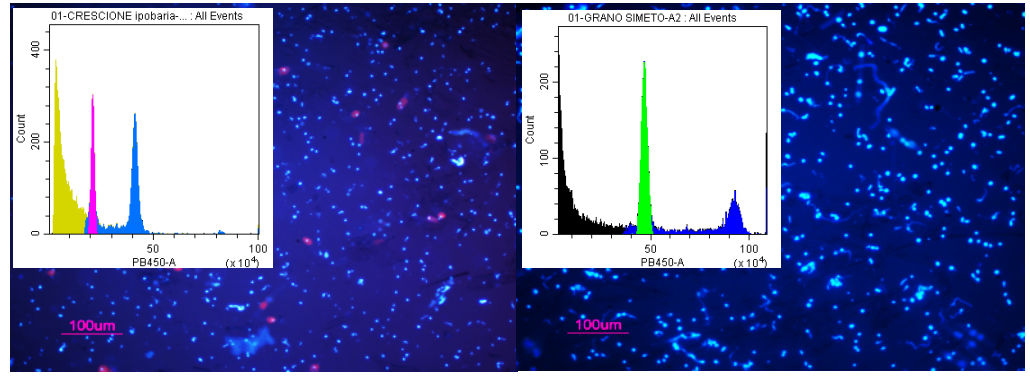

Fig2. Root nuclei suspensions at microscope (LB01+DAPI). A) *Lepidium sativum* B) *Triticum durum cv Simeto*

	Lepidium sativum	*Lepidium sativum*	*Triticum durum cv Simeto*	*Triticum durum cv Simeto*	*Triticum durum cv Simeto*	*Triticum durum cv Simeto*	*Triticum durum cv Simeto*	*Solanum lycopersicum cv Money Maker*
Extraction Buffer	LB01	LB01	LB01	LB01	LB01	LB01	LB01	LB01
Fluorochrome	DAPI	DAPI	DAPI	DAPI	DAPI	DAPI	DAPI	DAPI
Plant organ	LEAF	ROOT	ROOT	ROOT	ROOT	ROOT	ROOT	LEAF
Quanity	1mg	1mg	<1mg	1mg	2mg	4mg	10mg	1mg
Nuclei events	5574	4143	1504	3171	2237	1409	1278	4621
% debris	41%	77%	85%	68%	78%	86%	87%	47%
% nuclei	59%	23%	15%	35%	22%	14%	13%	53%
cv G1 (PB450-A)	7%	6%	4%	6%	5%	4%	9%	7%

Tab1. Summary data of the run analysis

Conclusions

Our FCM results show that this new method could be fast, reliable and cost effective. A number of analysis can be performed on a wide range of plant species and different plant organs resulting in a good quality of data. In this way, it's possible to analyse larger populations of plants and seedlings of interest, in respect to the world wide used chopping procedure spread in almost each plant flow cytometry labs (**Galbraith et al. 1983**).

References

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