Flow cytometry
(ploidy determination, cell cycle analysis, DNA content per nucleus)

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1.   General considerations and theory of flow cytometry

During recent years, flow cytometry has established itself as a useful, quick novel method to determine efficiently, reproducibly and at a reduced cost per sample the relative nuclear DNA content and ploidy level of a large number of species (plants and animals), that has also been used to sort cells with different traits from a mixed cell population (e.g. as when separating heterokaryons from parental protoplasts in protoplast fusion experiments for somatic hybrid production) and, more recently, for the analysis of the composition in various chemical components of different tissues (such as apoptotic markers bound to cell compartments). This chapter will focus on the protocols and uses of flow cytometry applied to *Medicago truncatula*.

Basically, a flow cytometer is a fluorescence microscope which analyses moving particles in a suspension. These are excited by a source of light (U.V. or laser) and in turn emit an epi-fluorescence which is filtered through a series of dichroic mirrors (Figure 1). Then, the in-built programme of the equipment converts these signals into a graph plotting the intensity of the epi-fluorescence emitted against the count of cells emitting it at a time given.

Thus, a flow cytometer consists of *fluidics*, *optics* and *electronics*, as it measures cells in suspension that flow in single-file through an illuminated volume where they scatter light and emit a fluorescence that is collected, filtered and converted to digital values for storage on a computer (Robinson, 2006).
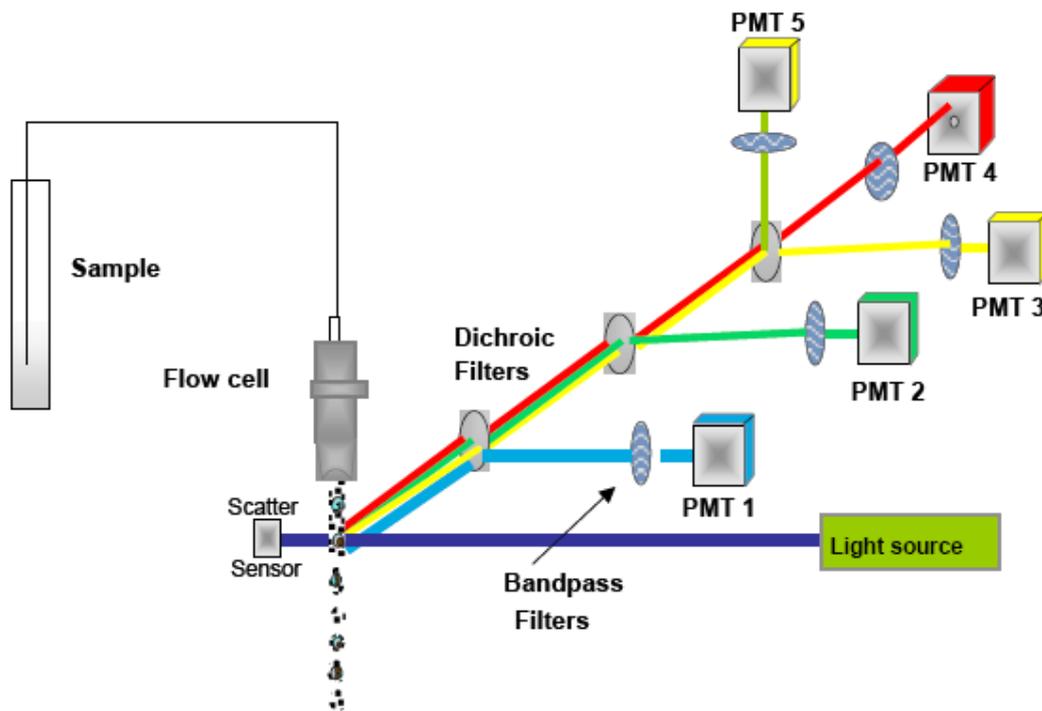
Figure 1. Simplified scheme of a flow cytometer (from Murphy, 2006).

2. Optics, measurements, fluorescent dyes, signal processing

In terms of fluidics, the string of cells in suspension flowing in a single-file is accomplished most frequently by injecting the samples into a sheath fluid as they pass through a small orifice (50-300 µm). Then, when the conditions are right, the sample fluid flows in a central core without mixing with the sheath fluid, and the process is termed "laminar flow". The introduction of a large volume into a small volume in order that it "focuses" along an axis is termed "Hydrodynamic focusing" (Figure 2).
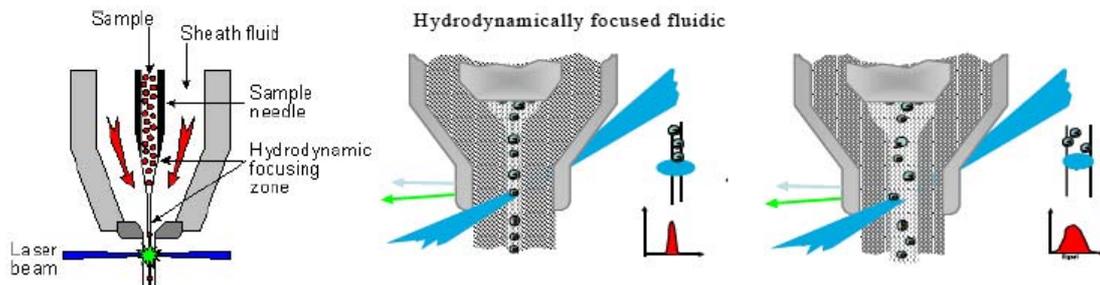

Figure 2. Basics of fluidics in flow cytometry. From left to right, flow chamber, a hydrodynamically focused fluid and an unfocused fluid where the increase of pressure widens the core and increases turbulence (from Murphy, 2006).

In terms of optics, optical signals are generated by the intersection of a light beam with cells or cellular compartments. Two types of light sources can be used, lasers (gas, acid state or diodes) and/or Arc-lamps (mercury or xenon), whereby two different kinds of optical phenomena can be detected by a photomultiplier tube: fluorescence or scattered light. Thus,

epilumination by Arc-lamps provides a mixture of wavelengths that must therefore be filtered to select only the desired wavelengths, i.e. it provides incoherent light, but they are inexpensive air-cooled units (Robinson, 2006). Conversely, lasers are monochromatic (i.e. of a single wavelength) and provide coherent light, traditionally from an argon gas source with excitation at 488 nm, but are much more expensive than an UV light source.

For the fluorescence to be detected by the photomultiplier, the cells have to be labelled with an appropriate fluorescent molecule whose properties will change on binding to nucleic acids, and Table 1 below gives the wavelengths of various commonly used fluorescent dyes after such binding.

Table 1: Some of the fluorophores (fluorochromes) used to label nucleic acids (the two most frequently used ones with each kind of light source are underlined in yellow).

Fluorophore	Excitation maxima (nm)	Emission maximum (nm)	Laser line (nm)
Propidium iodide	495, 342	639	488 A
Ethidium bromide	493, 320	637	488 A
Acridine orange	503	530 (DNA) - 640 (RNA)	488 A
Mithramycin	445	569	457 A
Chromomycin A_3	430	580	457 A
Hoescht 33342	395	450	UV A or K
DAPI	372	456	UV A or K
Pyronin Y	545	565	530 K 514 A
Thiazole Orange	509	533	488 A
Thioflavin T	422	487	457 A
$DiOC_1[3]$	482	510	488 A
YOYO-1	491	509	488 A
TOTO-1	514	533	514, 488 A
TOTO-3	642	660	630 H

In fact, the key feature of DNA probes is that they are stoichiometric, whereby the number of molecules of the probe bound is equivalent to the number of molecules of DNA found. Hence, when DAPI for instance binds to the A-T bases of DNA, the intensity of the fluorescence emitted will reflect the number of bounds and therefore also the DNA content in such DAPI-labelled nuclei.

Finally, in terms of electronics, the excitation light interacts with the stained particle that will emit a fluorescence signal which, after being transferred via the optical system of the flow cytometer to the photomultiplier, will be converted from analog to digital, and processed by the computer to produce a one-parameter or a 2-parameter dot-plot histogram.

A special attention must be given to the preparation of the plant material for the analyses. Several methods exist for this, dependent on the brand and model of the flow cytometer used. However, they all share some common steps, as schematised in Figure 3 below. First, a small quantity of plant tissues (a single foliole of an in vitro plantlet of *M. truncatula* may suffice) are roughly chopped (1-2 mm side pieces or strips) in an appropriate buffer to isolated the nuclei. Various methods exist that are based on use of either a single-step isolation + staining buffer (as in Figure 3) or a succession of a small volume (i.e. 400 µl) of nuclei extraction buffer, followed by a larger volume (1.6-2.0 ml) of the staining buffer. Secondly, not only nuclei but also various debris (chloroplasts, mitochondria, …) and soluble substances (phenolics, DNAse, RNAse, etc) from the cytoplasm and vacuoles are obtained in the suspension resulting from this chopping of the material. Possible ways of getting rid of such debris include washing the nuclei, blocking the unwanted signals or blocking the unwanted substances, but the most frequently used by far is the filtration of the suspensions obtained after chopping through a fine nylon mesh (20-100 µm pore diameter). Unfortunately,

sometimes this is not enough and, then, a modification of the composition and/or pH of the buffer systems and particularly of the staining buffer may be needed (i.e. modifications of the fluorescent dye concentration which, when too low results in understaining, and when too high in quenching; addition of proteinase K to deal with fading; …)
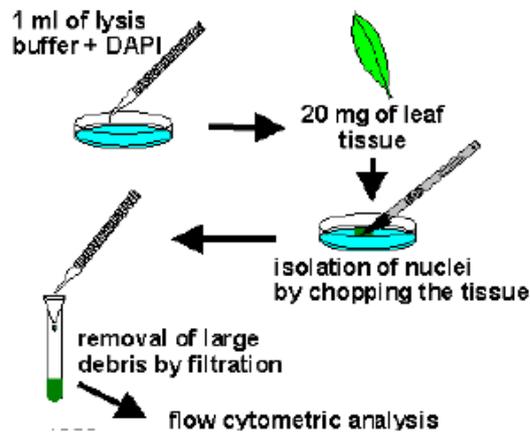

Figure 3. Scheme of the preparation of plant materials for flow cytometry analyses.

3.   Data collection and analysis

In flow cytometry, a parameter is a measured property of the particles, frequently a synonymous to an optical channel (e.g. it could refer to the fluorescence parameter used for DNA analysis whose number will depend on the number of optical detectors with which the flow cytometer is equipped). Thus, a one-parameter histogram displays the distribution of cell contents or, in other words, how many cells contain a given quantity of DNA or bind a given number of antibody molecules. In it, such cell content is assigned to one of many classes or channels and is represented on the x-axis, whereas the number of cells being assigned to a given channel is referred to as channel content or simply count and is shown on the y-axis. All cells having about equal quantities of the cell content, e.g. DNA, form a peak. For a typical DNA histogram one peak represents the G1 and another (with twice the channel value) represents the G2/M phase of the cell cycle (Figure 4). In studies of immunolabelling there is usually one negative peak (for unlabelled cells) and another one for labelled cells (positive). Such peaks can either be analysed using the (mostly) in-built computer of the flow cytometer, or they can be analysed by drawing ranges or numerical fits to know the mean intensity or the number of cells in a peak. On the other hand, in a correlated 2-parameter dot-plot, quantities of the cell properties (such as forward and side scatter intensity) are assigned to channels on the x- and y- axes, where each cell with a given intensity is represented by one dot in the dot-plot. Hence, several cells with the same combination of properties (e.g. forward and side scatter intensity) will share the same dot location and subpopulations of cells with similar properties will appear as clusters (that may be represented by a colour, cf. Figure 4) which can be circled by drawing lines around them (gating) to analyse the number of cells in each cluster.
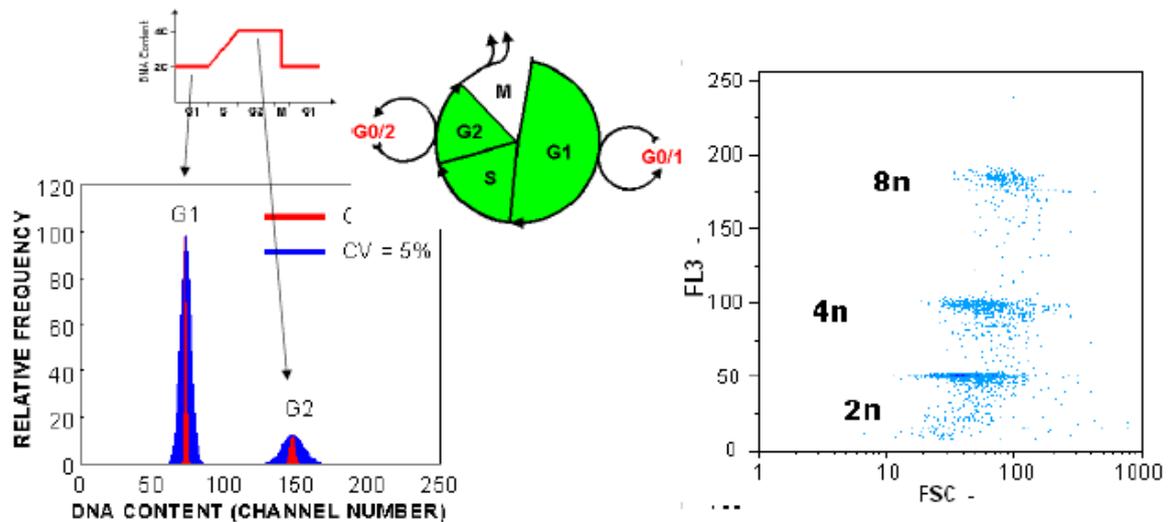
Figure 4 : Typical one-parameter (left) and dot-plot (right) flow cytometry profiles.

Two technical instrument settings are of paramount importance to obtain workable flow cytometry data from analysed samples. The first is the adjustment of the lower threshold (L-L) to avoid the acquisition of small and unwanted "noise" signals below it and therefore to allow the system to have more time for the particles of interest. So, measurements should always be started with a L-L as high as possible until no more of the small "noise" signals appear in the histograms but low enough not to remove signals from particles of interest. The second is the speed as for high accuracy measurements a low speed (of around 2 µl/sec) is advisable. In this respect, if the speed is increased too much, peaks in the histograms may become wider as a result of decreasing accuracy. On the contrary, if the speed is too low, particle sedimentation effects can influence a counting result.

The distribution of the nuclear DNA content within a cell population is obtained by comparing the number of cells in the different peaks or clusters, since the absolute DNA content is irrelevant. Moreover, particular care should be taken to the developmental stage of the analysed cells, as this may alter the quality of the analysis of the cell cycle performed. Also, it is crucial to include an internal standard, as a control, when attempting to analyse the ploidy level of G1-cells by comparing the positions of the first peak in profiles from different genotypes. Frequently used internal standards are chicken erythrocytes, UV Teflon beads, salmon sperm cells or, more simply, a suspension of nuclei of a genotype such as the mother plant (when comparing populations of in vitro regenerants), both parental genotypes (when analysing putative hybrids), or a model system where the relative DNA content per nucleus is well known and reliable among different genotypes within the species (i.e. pea, rice, tomato, and *M. truncatula*) for a given source tissue (leaf material for instance).

So, what kind of information can one recover from flow cytometry profiles ?

Firstly, and the most frequently reported use of flow cytometry in the literature has been to analyse the ploidy level of individuals obtained following experiments of haplo-diploidisation or chromosome doubling (Ochatt et al, 2005). Unfortunately, there are no successful examples of production of double haploids after andro or gynogenesis of grain legumes in the literature so far and, hence, this tool is still to be tested for that goal with this group of species. Examples exist, though, on the production of tetraploids following chromosome doubling in various grain legume crops. Thus, an internal standard was run together with nuclei suspensions of the genotypes analysed and the position of the G1 peak used to establish the ploidy level of such material. In this respect, as explained above, the mean of the first peak

will appear at double the intensity of the epi-fluorescence emitted by nuclei of the double population compared to the original one.

A second use of flow cytometry is for the analysis of the cell cycle in the nuclei and of the division frequency, expressed through the mitotic index (MI), of the cell population studied. Figure 5, depicts a typical flow cytometry profile for an euploid genotype of *M. truncatula* and is analysed below.
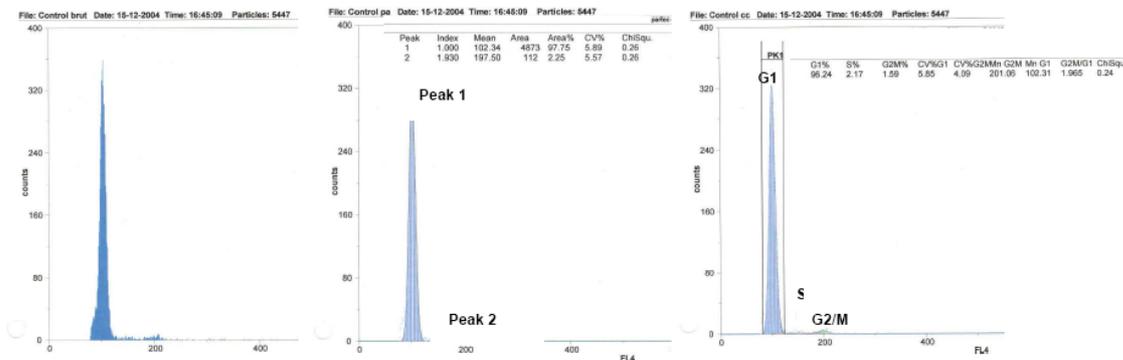

Figure 5. Flow cytometry analysis of a seedling plant of M. truncatula A17. From left ro right, the raw profile, peak analysis and cell cycle analysis of a population of 5447 nuclei from leaves.

The mitotic index is calculated, according to the formula,

$$MI = 4 \times 4C / \Sigma\, 2C + 4C$$

where 2C and 4C correspond to the mean value of the first (nuclei in G1 phase) and second peaks (nuclei in G2/M phase) in the profiles obtained, respectively. For normal division, MI = 2.000. Very small reductions or increases of the value of the MI indicate problems with cell division and, concomitantly, in the cell cycle (as will be discussed below).

Thereafter, the mean C value of the sample (i.e. the value normally found in the literature) is calculated according to the formula:

$$Mean\ C\ Value = \frac{\Sigma^{n}_{i=1} C_I \times N_I}{N_{sample}}$$

where N: number of peaks of DNA content in the sample ; C_i: C value of nuclei in peak n_i; N_i: number of nuclei in peak n_i; N_{sample}: number of nuclei in all peaks of the sample.

A third use of flow cytometry is, as discussed above, to measure the cell content of various subcellular substances (such a cell wall fractions, apoptotic markers, other dye-labelled substances capable of emitting an epi-fluorescence when excited by the UV or laser light sources of the equipment). One such example will be briefly described in this chapter.

4.   **Uses of flow cytometry for the characterization of *Medicago truncatula* tissues and plants**

Within the context of the EU-funded Grain Legumes Integrated Project, GLIP, a rather large population of regenerants of a *Tnt1* insertion mutant of *M. truncatula* genotype 2HA has been generated and also analysed in terms of flow cytometry, number of copies of the transgene, etc. In this respect, retrotransposons are known to be capable of adding to each other in the

genome and thereby increasing the relative DNA content per nucleus of individuals. It was therefore deemed important to analyse this population (as well as that of primary transformants of this genotype obtained after *Agrobacterium tumefaciens*-mediated gene transfer and harbouring the retrotransposon) in order to check whether this had influenced their relative nuclear DNA content, but also to characterize them in terms of true-to-typeness, and recent work in our group was aimed at this goal. Indeed, flow cytometry is a powerful tool for this as very early on the newly obtained regenerants/transformants can be analysed reliably and using a very reduced amount of tissues. Against this background, literature concerning the flow cytometric analysis of *Medicago* plants produced in vitro is rather scanty (Iantcheva et al, 1999; Elmaghrabi & Ochatt, 2006; Ochatt et al, 2005).

The following paragraphs will summarize the work undertaken in our laboratory to analyse different tissues of various genotypes of *Medicago truncatula* (A-17, J5, 2HA, insertion mutants of 2HA carrying the *Tnt1* retrotransposon from tobacco, and the hypernodulating mutants TRV25 and TR122 [Sagan et al, 1995]).

For flow cytometry analysis, a Partec PAS-II flow cytometer equipped with an HBO-100 W mercury lamp and a dichroic mirror (TK420) was used. The DNA content and ploidy level of calluses and cell suspensions (organogenic, embryogenic and non-regenerating) cultured in vitro and of leaves, harvested from regenerated shoots in vitro and from regenerated plants in the greenhouse, was compared with leaf controls from seed-derived plants of the various genotypes. Nuclei were mechanically isolated by chopping the tissues with a razor blade in 400 µl of nuclei extraction buffer and 1.6 mL of staining buffer (Partec®) containing 4,6 diamidino-2-phenylindole (DAPI), and the suspension was filtered through a 50 µm mesh (Elmaghrabi and Ochatt, 2006). A normal (euploid) profile consists of two peaks, corresponding to the nuclei in the G1 phase of mitosis (2C DNA) and those in G2/M (4C DNA). For calculations of DNA content, pea or *Medicago truncatula* leaf tissues were used as an internal standard (i.e. run simultaneously with the material analysed), since the DNA content measured is more a magnitude than an accurate content. All flow cytometry assessments were repeated at least three times at monthly intervals for each sample analysed and a minimum of 2500 nuclei per run (i.e. 7500 per sample) were counted, the cell cycle was analysed and the mitotic index and mean C value were calculated, according to the formulae above.

A first series of experiments was run with materials with a different competence for regeneration in vitro, to check them for ploidy stability, to screen for "x-ploids" and to detect eventual aneuploids and chimaeras. The data in Figure 6 summarize the range of results observed within this population.

Figure 6a-f shows typical flow cytometry profiles of the major classes of materials produced in vitro with barrel medic. Thus, the most frequent, true-to-type regenerants, exhibit a two-peak profile (as described above) and coupled with a MI of 2.0 (Fig. 6a). The second most common category of regenerated material concerns the mixoploid calluses. In this case, taking a 2n/4n regenerated individual the profiles are composed of three peaks where the first one corresponds to G1 nuclei of the 2n cell subpopulation, the second peak to the summation of nuclei in G2/M of the same subpopulation plus those in phase G1 of the 4n subpopulation of cells, and the third peak corresponds to the nuclei in phase G2/M of mitosis of the subpopulation of cells of the higher ploidy level (Fig. 6b). Such material, generally, does not give rise to viable, fertile plants in vivo. Several other classes of tissues can be obtained in vitro and some are also depicted in Figure 6c-f. These include endoreduplicated (commonly observed for calluses and cell suspensions but also for differentiated tissues *in planta* as will be discussed later), aneuploid, senescent and very abnormal tissues. The typical flow cytometric imprint of an endoreduplication phenomenon consists of profiles with a sequence of peaks of progressively decreasing magnitude (Fig. 6c), which appear as a consequence of

the occurrence of a succession of endomitotic DNA replication processes whereby nuclei become bigger and bigger and do not undergo karyodieresis. Such a process leads to the blockage of regeneration competence from such calluses (Ochatt et al, 2000). Another, not very frequent yet observable, kind of profile is that of aneuploid tissues whose cytometric imprint is typically the production of a "shouldered" (split) peak (Fig. 6d). This may concern either the G1, the G2/M or both peaks in the profile and the precise chromosome complement of the material in question must obviously be confirmed by (root tip or other) chromosome counts before a final conclusion may be drawn. This class of callus has seldom been observed to produce viable regenerants. Another class of profiles is that where one peak (the G1 nuclei) only is apparent (Fig. 6e), and it corresponds to the senescent tissues where divisions proceed no more. This type of profile is very frequent when "old" plants and tissues (not subcultured on time) are analysed. The last profile shown in Figure 6f is just an example of a quite infrequent situation, where a combination of abnormalities is found in a single piece of undifferentiated tissue and does not lead to the recovery of regenerated shoots, embryos of buds. In this example, the onset of endoreduplication can be observed as from the 4C DNA level and is coupled with the occurrence of mixoploidy (2n/4n).
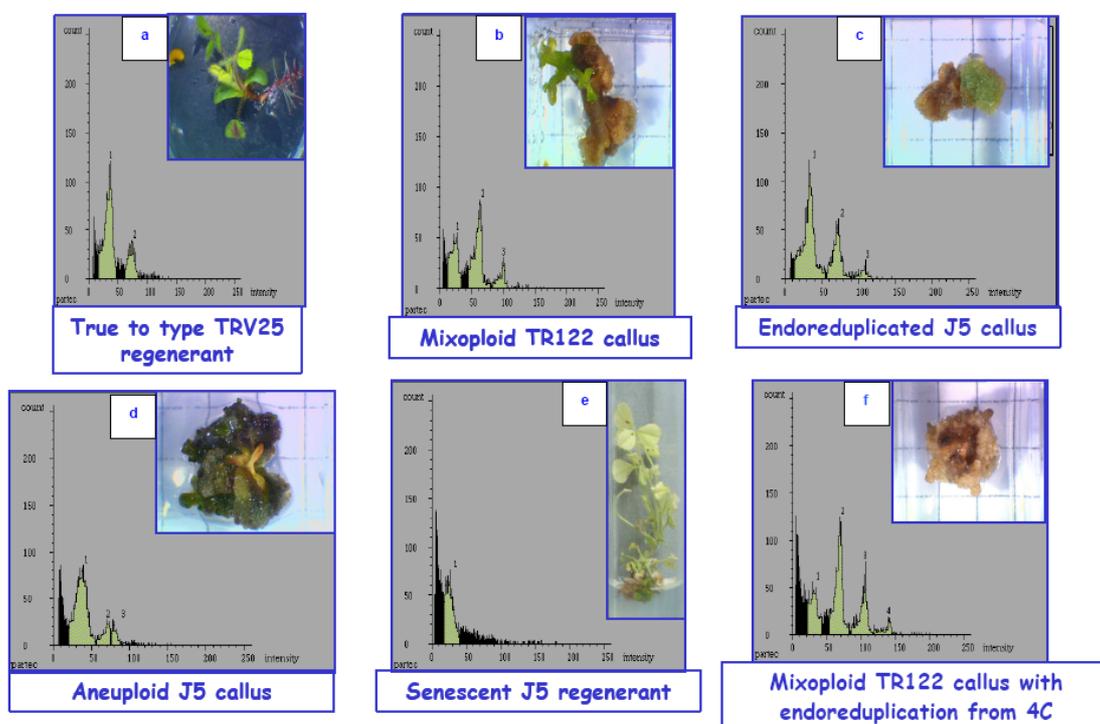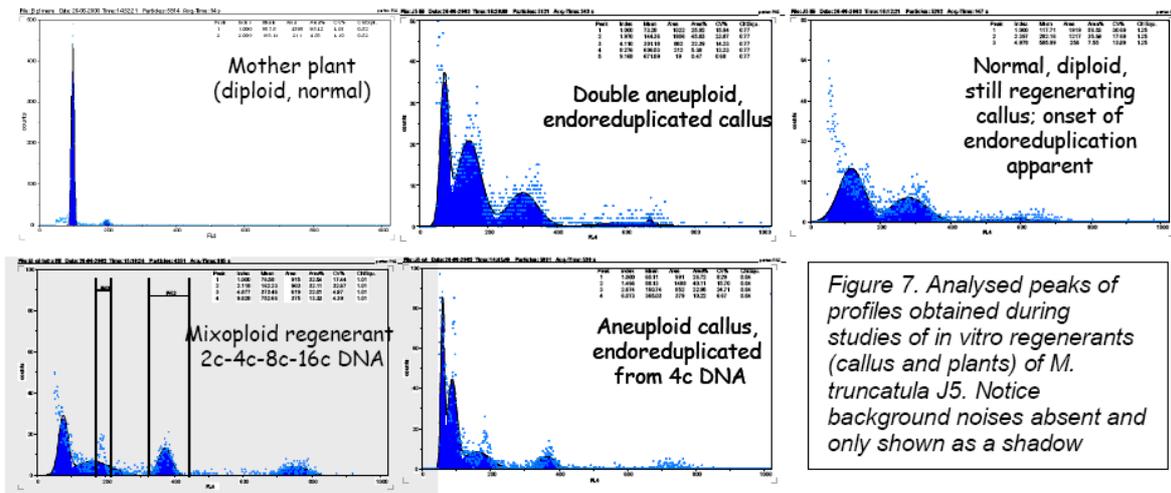

Figure 6. The range of flow cytometry raw profiles and corresponding phenotypes of various in vitro cultured tissues and plants of several M. truncatula genotypes. Notice background noise in black.

When analysing similar profiles after getting rid of all background noises, these profile classes are more evident, as shown in Figure 7, where the MI analyses of such materials also becomes possible.

As part of an independent series of studies, we undertook the unravelling of the competence for conversion of somatic embryos to plants with this species, which sometimes can be problematic but also, when successful, lead to the recovery of sub-fertile and sometimes even sterile plants. For this, we based our work on the analysis of hyperhydric regenerated plants and tissues, previously shown to be a possible reason of such fertility problems with in vitro-produced plants of the related grain legume grasspea (*Lathyrus sativus* L.) (Ochatt et al, 2002). Thus, both seed-derived plantlets of A-17 (control), hyperhydric and normal plantlets

regenerated from calluses and cell suspensions via embryogenesis, and developing and blocked somatic embryos were analysed by flow cytometry.

Figure 7. Analysed peaks of profiles obtained during studies of in vitro regenerants (callus and plants) of M. truncatula J5. Notice background noises absent and only shown as a shadow

Confirming our previous observations with grass pea (Ochatt et al, 2002), the hyperhydric plantlets mostly gave flow cytometry profiles including three peaks of a decreasing magnitude (indicative of an onset of endoreduplication and systematically coupled with non viable plants in the greenhouse) and only seldom were normal two-peaked profiles obtained (and always for those plantlets where hyperhydricity symptoms were very light and could be reverted upon transfer to a hormone-free medium prior to in vivo acclimatization). Interestingly, both the non-developping somatic embryos and those that only gave abnormal, sub-fertile or sterile plants in the greenhouse always gave flow cytometry profiles with three peaks, characteristic of the onset of an endoreduplication phenomenon, and similar to those observed with protoplast-derived calluses of pea when they were not capable of regenerating normal, fertile plants (Ochatt et al, 2000). Figure 8 summarizes these results, which were identical irrespective of the non-differentiated tissue sources for the embryos analysed (i.e. calluses or cell suspensions) (Elmaghrabi & Ochatt, 2006).
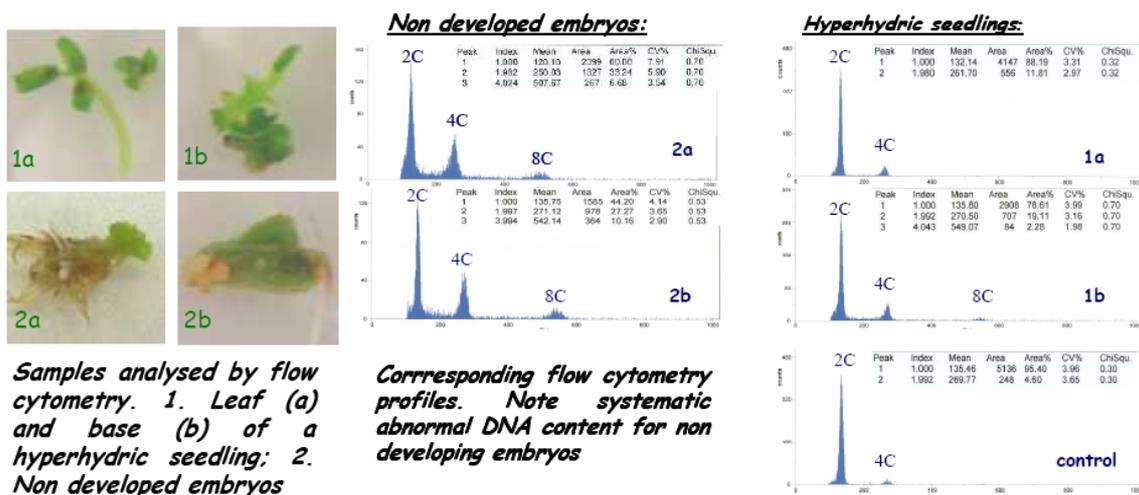

Samples analysed by flow cytometry. 1. Leaf (a) and base (b) of a hyperhydric seedling; 2. Non developed embryos

Corrresponding flow cytometry profiles. Note systematic abnormal DNA content for non developing embryos

Figure 8. Flow cytometry profiles of non-developing hyperhydric plantlets (1a, 1b), somatic embryos (2a, 2b) and a seedling (control) of M. truncatula A-17.

It is against this background that we analysed a rather large population of regenerants and primary transformants of *M. truncatula 2HA* carrying the retrotransposon *Tnt1* and also other primary transformants in both sense and anti-sense. Thus, from a total population of 906 individuals analysed the results were as detailed in Table 2.

Table 2. *Percentage of the different classes of flow cytometry profiles obtained after analysing a population of 906 independent regenerants from regeneration and Tnt1-Agrobacterium-mediated gene transfer of M. truncatula 2HA*

Flow cytometry Profile	Number of individuals	% of total population analysed
Control (seed-derived mother plant)		
Total	906	100.0
Normal, euploid, true-to-type	763	84.2
Off-type (Total)	143	15.8
Tetraploids	94	10.4
Mixoploids	30	3.3
Endoreduplicated	13	1.4
Aneuploids	4	0.5
Non-exploitable	2	0.2

The results obtained indicated that, provided the right media sequence and culture conditions are employed throughout, the percentage of non true-to-type plants regenerated remains reasonably low, and is compatible with the large-scale use of in vitro approaches for the generation of such populations of genotypes.

5.   Other uses of flow cytometry in *Medicago truncatula*

Apart from using flow cytometry to characterize in vitro regenerated plants and tissues, this approach can serve for the determination of the cellular and subcellular content of various other substances provided they can be stained with fluorescent dyes. Examples on this are very numerous in the literature for other plant species but scanty with *Medicago truncatula* or related grain legume crops.

Thus, one such use of flow cytometry concerns the timing of the different developmental phases during seed and embryo development, where endoreduplication is observed and used to explain this. As already discussed above, the phenomenon of endoreduplication is characterized by a modification of the mitotic cycle with DNA duplication but without mitosis, i.e. the nuclear DNA content is doubled at each cycle to pass from 2C to 4C, then 8C, corresponding to a succession of S phases, but without cell division. Different explanations have been sought for this, e.g. in their study, Lemontey et al (2000) have shown that a nuclear DNA content equivalent to 8C DNA (onset of the endoreduplication phenomenon) indicates the end of the division phase in cotyledonary cells. Previously, Gendreau et al (1997) had demonstrated that the phenomenon of endoreduplication precedes cell elongation in the hypocotyls of *Arabidopsis thaliana*. Thus, when such 8C peak appears very early on, it would imply the beginning of the embryogenesis phase resulting in a modification of cell shape, with elongating cells. It has often been stated in the original literature on this subject that the onset of embryogenesis in isolated cells is accompanied by the establishment of a bipolar type of growth, whereby cells grow in length as opposed to the iso-diametric form characteristic of non-embryogenic cells in active division (Ammirato, 1983). However, despite recent studies aimed at distinguishing between cell enlargement and cell elongation with *Arabidopsis* (Sugimoto et al, 2001) roots, no explanation to this process within embryos is available in the literature. In a recent study (Ochatt et al, 2006) we have tried to identify early indicators of the

acquisition of embryogenic competence from cell suspensions of various genotypes of *M. truncatula*. In this work, various cell morphometry traits were useful for this goal.

In parallel to this, the study of cell elongation requires an in depth knowledge of the activity of the enzyme xyloglucan-transglycosydase (XET) which permit cell expansion by favouring the integration of newly synthesized xyloglucans into the cell wall (Vissenberg et al, 2000). A mixture of hydrophilic xyloglucan oligosaccharides (XGO) whose major components are nona-, plus octa- and hepta-saccharides is labelled with sulforhodamine (XGO-SR) (Fry et al, 1992), is recognized as the substrate acceptor by XET. Such mixture is then diluted in the CIM medium (Trinh et al, 1998) with the pH adjusted to 5.8, to obtain a solution at 6.5 µM. Then, equal volumes of cells in suspension and of XGO-SR are added simultaneously, then incubated for 72 h in dark with slow shaking. Cells are rinsed with 1 ml of a ethanol/formic acid/water (15:1:4 [V/V/V]) solution for 10 min to eliminate the non-reactive XGO-SR, which are soluble in this solvent. A further overnight incubation in 5 % formic acid also permits to eliminate the XGO-SR non linked to the wall (i.e. soluble in the apoplast). Cells are then excited with a green light at 540 nm and the fluorescence is visualized under UV. Then, the incorporation of the XGO-SR in the wall produces a red-orange fluorescing polymer at the cell wall (Figure 9), indicative of the presence of an active XET and not of inactive pro-enzymes. Such fluorescence is only emitted by embryogenic cells.
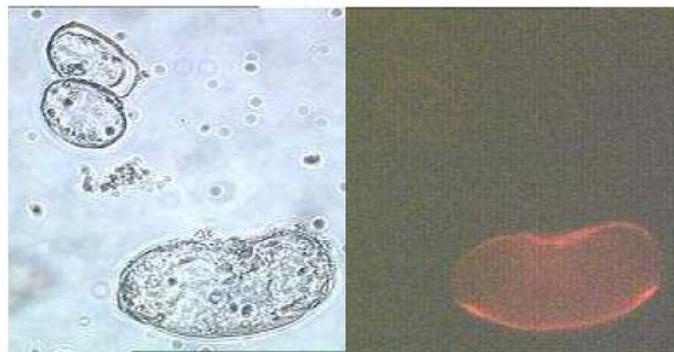

Figure 9. Detection of an active XET in the walls of suspension cultured cells of M. truncatula TR122. The same microscopic field observed under transmission (left) and UV (right) light.

In this context, one additional use of flow cytometry, and a much less known of, is for the determination of the hemicellulose content of cell walls. For this, seed samples are prepared as already indicated for leaf tissues and, once materials have been chopped and sieved, 1 ml of a solution containing 30 µg/ml of sulforhodamine 101 (SR101) in 0.2 M Tris-HCl buffer at pH 7.4, plus 0.2 M NaCl is added to the filtrate following the method described by Ulrich (1992). The samples are then excited with an argon laser lamp and the emitted fluorescence is measured. In this respect, SR101 absorbs at a wavelength of 586 nm and emits a red fluorescence at 605 nm, with results expressed graphically through the number of particles counted at each intensity of fluorescence emitted.

Figure 10 depicts the results typically obtained with this sort of studies, where a profile including three zones is obtained for the control (i.e. in this case leaves of *M. truncatula* J5), whereas for non-differentiated tissues the profiles obtained include four distinct zones. Thus, in leaves of J5 analysed the first peak represents 15.65 % of the cell population and includes cells with a very low hemicellulose content, a second sub-population (19.9 % of the total) concerns those cells with an intermediate hemicellulose proportion in their walls, while a third subpopulation can be identified with a high content of hemicellulose in the walls and accounting for 55 % of the total counted. Conversely, when cells from calluses or cell

suspensions are analysed a fourth sub-population, accounting for 16 % of the total particles counted is apparent and it translates those cells with the highest hemicellulose content in the walls. These observations have been confirmed on a very large number of non-differentiated tissues analysed compared to leaf tissues.
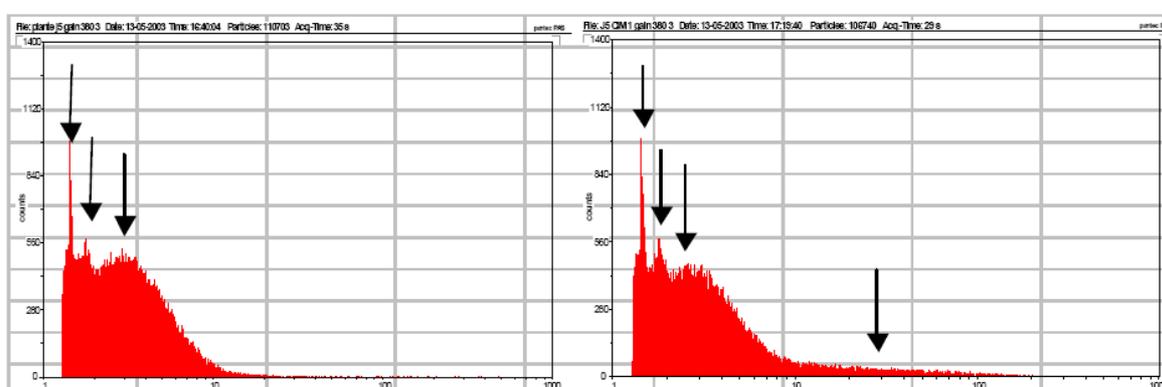

Figure 10. Flow cytometry analyses of argon laser excited samples of leaf (left) and callus (right) tissues of M. truncatula J5 labelled with sulforhodamine 101. The different cell sub-populations, in terms of hemicellulose content in the walls are arrowed.

The summation of these findings makes of flow cytometry an interesting tool for the early prediction of both the regeneration competence from undifferentiated tissues and also of the further fertility of the regenerants obtained. These uses add to the better known utilisation of flow cytometry for the characterization of plants, tissues and regenerants in terms of ploidy level, of nuclear DNA content and of division frequency (through the detailed analysis of the cell cycles).

6.   References

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