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Flow cytometric analysis of the cell cycle in different coconut palm (*Cocos nucifera* L.) tissues cultured in vitro

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Abstract We conducted a study of the cell cycle of coconut palm tissues cultured in vitro in order to regulate regeneration. Coconut palm is a plant for which it is difficult to monitor the ability of the meristematic cells to actively divide. Cell nuclei were isolated from various types of coconut palm tissues with and without in vitro culture. After the nuclei were stained with propidium iodide, relative fluorescence intensity was estimated by flow cytometry. Characterization of the cell cycle reinforced the hypothesis of a block in the G_0/G_1 and G_1/S phases of the coconut cells. A time-course study carried out on immature leaves revealed that this block takes place gradually, following the introduction of the material in vitro. Synchronization of in vitro-cultured leaves cells using 60 μ*M* aphidicholin revealed an increase in the number of nuclei in the S phase after 108 h of treatment. The significance of these results is discussed in relation with the ability of coconut tissue cultured in vitro to divide.

Keywords Cell cycle · Meristematic potential · Flow cytometry · Tissue culture · *Cocos nucifera* L.

Introduction

The mastering of plant in vitro regeneration starting from primary explants taken in vivo relies on controlling expression of the "meristematic potential" or aptitude of the tissues for cell division. In vitro organogenesis is usually categorized into various stages: a dedifferentiation stage, in which cells of the primary explant acquire the ability to actively divide; a more or less transient cell-proliferation stage, leading to callus formation; and a stage during which organogenesis ability is acquired, where cells or groups of cells will be determined for the differentiation of a specific organ (Georges and Sherrington 1984; Sugiyama 1999). In most plant species, this succession of stages depends on the application of growth regulators, notably auxins and cytokinins (Ammirato 1985; Komamine et al. 1990) but also on the ability of tissues to respond to hormone treatments during the different stages of in vitro organogenesis. However, there are species—for example, soybean, cocoa, cotton—for which in vitro regeneration is particularly difficult to achieve (Georges and Sherrington 1984). Included in this latter group is the coconut palm, despite the fact that it has been the focus of much work since the 1970s. While the regeneration of whole coconut plants from different explant sources (immature leaves and inflorescences, plumules) has been reported (Branton and Blake 1983, 1986; Raju et al. 1984; Pannetier and Buffard-Morel 1986; Buffard-Morel et al. 1988, 1992; Verdeil et al. 1994; Chan et al. 1998), coconut remains a recalcitrant species with respect to in vitro culture. This recalcitrance is reflected mainly in the slowness of in vitro morphogenesis (it takes 4–6 months to obtain callus from immature leaves), in a low mitotic index (Jesty and Francis 1992)—even in tissues containing numerous meristematic cells—and in the presence in cultured tissues of small cell nuclei with condensed chromatin (Verdeil et al. 1994). These events reflect the difficulty in controlling the orientation of coconut palm cells towards a meristematic state (cells starting active divisions).

Plant morphogenesis involves both the tight control and the co-ordination of proliferative activity through control of the cell cycle in meristematic tissues (Sugiyama 1999; Planchais et al. 2000). The cell cycle can be classified into four steps: the S phase where DNA replication takes place, which is preceded by the G_0/G_1 phase and followed by the G_2 phase and mitosis (M). The repartition of cells in the different phases of the cell cycle can be a good indicator of cell-division activity for a tissue culture in vitro (Winkelmann et al. 1998; Yan-

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paisan et al. 1999). We therefore decided to examine recalcitrance to in vitro culture of coconut palm tissues by studying the distribution of cells in the different phases of the cell cycle. This was accomplished by measuring cell nuclei DNA content by means of flow cytometry.

Flow cytometry is a rapid and efficient method for estimating the DNA content of large populations of cells (Fox and Galbraith 1990; Brown et al. 1991; Dolezel 1991; Marie and Brown 1993) and has been proven suitable for estimating the position of cells within the cell cycle with respect to the total cell population (Galbraith et al. 1983; Fox and Galbraith 1990; Planchais et al. 2000). Flow cytometric studies on coconut cells have not been described to date.

In the investigation reported here, we compared different types of tissues cultured in vitro: immature leaf explants, slow-growing calli (SGC), fast-growing calli (FGC) and shoot meristems excised from embryos and cultured in vitro. Apart from the FGC, on the whole the tissues studied were characterized by a high percentage of cells in the G_0/G_1 phase. A kinetic study carried out before and after the leaf explants were placed in in vitro culture showed that this high accumulation of cells in the G_0/G_1 phase was linked to the culture conditions. A study of cell synchronization using aphidicholin revealed that only 20% of cells could be synchronized.

On the basis of these data, we put forward the hypothesis that the difficulty in controlling the potential for cell division of coconut tissues in vitro would seem to be associated with a blockage of cells in the G_0/G_1 phase linked to culture conditions.

Materials and methods

Plant material

We used four types of coconut tissues from the self-fertilizing variety Malayan Yellow Dwarf (MYD) cultured in vitro. Immature leaves and zygotic embryos were obtained from the CNRA station in the Ivory Coast.

Immature leaves

This type of tissue described by Buffard et al. (1988, 1992) is primarily composed of differentiated cells, but there are also meristematic cells located at the periphery of the vascular bundles (perivascular cells at the origin of the calli). The immature leaves were recovered after dissection of the spear (or palm heart) located in the center of the leaf crown. Following disinfection in a sodium hypochloride solution (3.5% active chlorine) for 20 min and a 5-min rinse in sterile water, the spear was cut into small 1-cm-wide sections comprising fragments of immature leaves. Flow cytometry analysis was carried out before and after inoculation of the leaf tissues on a callogenesis medium.

Shoot meristems cultured in vitro

Shoot meristems were isolated from zygotic embryos and consisted of strictly meristematic cells surrounded by leaf primordia. The shoot apexes were recovered after the dissection of coconut zygotic embryos (Chan et al. 1998) and cultured on the same culture medium as that used for immature leaves. Flow cytometry analysis was carried after a 6-month culture period.

Slow-growing calli

SGC were obtained by culturing leaf explants following the protocol described by Buffard-Morel et al. (1992). These compact nodular SGC comprise a mass of differentiated and disorganized cells. Their growth was ensured by a peripheral meristematic zone organized in a pseudo-cambium (Buffard-Morel et al. 1992; Verdeil et al. 1994).

Fast-growing calli

This friable type of calli consisted of islets of active cells surrounded by actively dividing cells, which in turn were surrounded by more or less vacuolarized dedifferentiated cells. In coconut, unlike oil palm (Hanower and Hanower 1984), these calli appeared in a random manner at a low frequency (less than 1/25,000).

Culture media and conditions

The shoot meristems were cultured on a medium containing Eeuwens macro- and micro-elements (1976) and 2 g l^{-1} Morel and Wetmore vitamins (1951), without hormones, and supplemented with 30 g l^{-1} glucose and 2 g l^{-1} activated charcoal (C-5385; Sigma). The pH was adjusted to 5.0 before the charcoal was added. The medium was solidified with 4 g l^{-1} Gelrite (P-8169; Sigma) and autoclaved for 20 minutes at 112°C. Immature leaves were cultured on the callogenesis medium as described by Buffard-Morel et al. (1992).

The SGC and FGC were cultured in vitro on a medium containing Eeuwens macro- and micro-elements (1976), 2×10^{-4} *M* 2,4-dichlorophenoxyacetic acid (2,4-D), 3×10^{-5} *M* N^6-(3-methyl-but-2-enyl)adenine (2iP) 40 g l^{-1} sucrose and 1.25 g l^{-1} activated charcoal (C-5385; Sigma). The pH was adjusted to 5.8 before the charcoal was added. The medium was solidified with 3 g l^{-1} Gelrite (P-8169; Sigma) and autoclaved for 20 min at 112°C (Verdeil et al. 1994). All of the explants were cultured in the dark at 27°C (±1°C) and a relative humidity of 70% (±5%) (Buffard-Morel et al. 1988).

Influence of the duration of the tissue-culture period

For this experiment, we used immature leaves collected from adult coconut palms (leaf ranks F-3 to F-6, with F0 being the last fully opened leaf). The percentage of cells in the different phases of the cell cycle was determined before the start of in vitro culture and 1, 4, 7, 14, 28, 45 and 60 days after culture initiation.

Treatment with aphidicholin

This experiment was carried out on fragments of immature leaves transferred to 15-ml Falcon tubes containing 3 ml of culture medium prepared with aphidicholin (A0781; Sigma) and without activated charcoal. Three aphidicholin concentrations (30, 60 and 90 μ*M*) and three incubation times (72, 108 and 144 h) were tested. Incubation was carried out under the same culture conditions as for the shoot meristems. To release the aphidicholin, we washed the immature leaves thoroughly three times (10 min each) and allowed them to recover on an aphidicholin-free medium.

Flow cytometry

Sample preparation

Nuclei were extracted by chopping up the four types of tissues studied: immature leaves (2 cm^2), shoot meristems, SGC and FGC (approx. 500 mg). The material was chopped for 2 min with a razor blade in a Petri dish containing 2 ml of Dolezel et al. (1989) extraction buffer modified with Triton 10%. The suspension containing the nuclei was then filtered through a bolting-cloth (36-µm mesh size) to remove cell debris.

Nucleus staining

The nuclei were stained with propidium iodide (P4170; Sigma), an intercalating fluorochrome, by incorporating 100 µl of a propidium iodide stock solution (at 1 mg ml^{-1}) in 300 µl of filtered nucleus suspension. The saturating staining concentration used was 330 µg ml^{-1} (Barre et al. 1996). Following stirring in a vortex to separate nuclei clusters, the solution was left to incubate for 5 min before the samples were analyzed by flow cytometry.

Assessment of DNA content

A FACScan cytometer (Becton-Dickinson, USA) was used with an argon laser (15 mV) at 488 nm, encompassing an emission range of greater than 590 nm. Fluorescence (area and width) was collected on 1,024 channels. The cytometer was calibrated beforehand with Fluoresbrite calibration grade 2-µm beads (Polysciences) as described by Barre et al. (1996). Beads were used as an external standard and checked every four samples. The high voltage was set at 500 V and remained constant throughout the experiment. A minimum of 10,000 nuclei was monitored for each histogram. The data were processed with CELLQUEST software (FACScan Becton-Dickinson) and presented in histogram form. The fluorescence histograms were resolved into G_0/G_1, S and G_2 cell-cycle compartments with a peak-reflect algorithm using two Gaussian curves (MODFIT.LT, Verity Software, USA).

For the measurement of the absolute DNA value, diploid *Petunia hybrida* (PXPC6, INRA, Dijon) (2C=2.85 pg) and/or *Hordeum vulgare* (2C=7.27 pg) (Marie and Brown 1993) leaf nuclei were used as internal standards.

Experimental design

For each type of coconut tissue studied, the percentage of cells in the different phases of the cell cycle (G_0/G_1, S and G_2/M) was calculated from five replicates per sample. The experiment was independently replicated twice. The data were processed by ANOVA and means compared by the Newman (1939) and Keuls (1952) test. It was thus possible to determine treatments between which significant differences existed.

Results

The nuclear DNA content of coconut was estimated to be 5.6±0.2 pg DNA/2C using petunia and barley nuclei as internal standards; this is approximately 5.4×10^9 bp according to the conversion factor given in Marie and Brown (1993). This was the mean of 20 replicates.

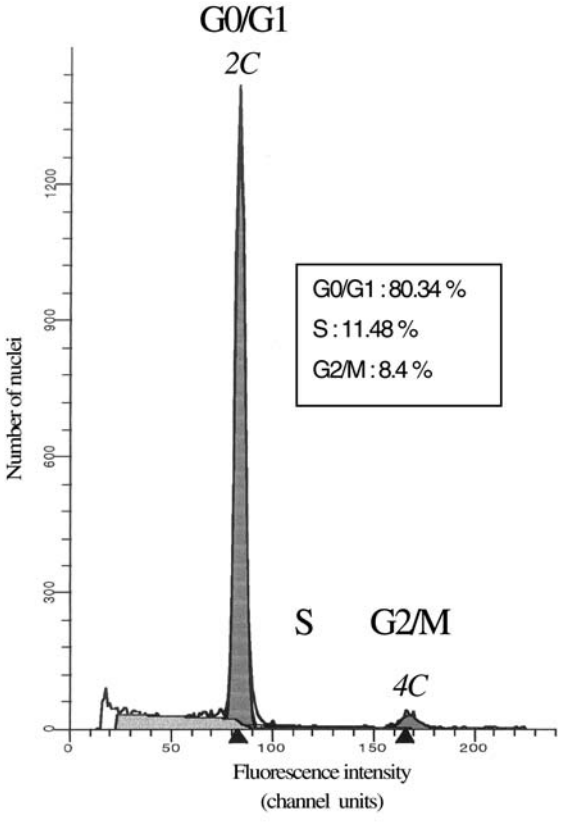

Fig. 1 Histogram of fluorescence intensity of nuclei isolated from coconut palm immature leaves after staining with propidium iodide. The fluorescence pulse was connected on a 1,024-channel analyzer. Results represent measurements of about 10,000 individual nuclei. The coefficient of variation was 3.4%. The fluorescence histograms were resolved into G_0/G_1, S and G_2 cell-cycle compartments with a peak-reflect algorithm using two Gaussian curves (Modfit.LT, Verity Software, USA). DNA content is expressed in arbitrary units

Comparative study of nucleus distribution in the different phases of the cell cycle with respect to different tissue types

Figure. 1 shows the distribution of the cell population of immature coconut leaves over cell-cycle phases. The distribution of the nuclei extracted displayed a prominent peak at 2C corresponding to nuclei in phase G_0/G_1 and a smaller peak at 4C corresponding to nuclei in phase G_2/M. Nuclei with a DNA content of between 2C and 4C are considered to be in phase S (DNA replication). This profile (Fig. 1) is representative of what was observed for shoot meristems, SGC and FGC. Coefficients of variation ranged from 3% to 6% for all of the analyses performed.

Nucleus distribution in the different phases of the cell cycle was dependent on the type of tissue considered (Table 1). As expected, the percentage of cells in phase G_0/G_1 was higher in SGC than in FGC (75.6% versus 66.8%). The percentage of nuclei in phase S was significantly higher in FGC (25.7%) than in SGC (16%).

More surprisingly, the percentage of cells in phase G_0/G_1 was higher in the shoot meristems (92%) than in the

Table 1 Comparison of the nuclei distribution in the different phases of the cell cycle as a function of different tissue type. Values are the means of five replicates. Results of one-way analysis of variance (ANOVA) are given: Snedecor's variable (F) and Type-I error (P) (*SGC*slow-growing calli,*FGC* fast-growing calli)

Type of material	Cell-cycle phases[a](%)[b]		
	G_0/G_1	S	G_2/M
Immature leaves[c]	80.3±4.8b	11.5±3.6b	8.4±1.9b
Shoot meristems	92±1.1a	3.7±0.9a	4.3±0.4a
SGC	75.6±1.5b	16.1±1.6b	8.4±0.6b
FGC	66.8±5.2c	25.7±6.6c	7.8±1.4b
ANOVA—F	37.86755	27.14147	12.12168
ANOVA—P	0.000000	0.000000	0.000096

[a] Values followed by the same letter are not significantly different at the 5% level as determined by the Newman and Keuls' test
[b] Percentage was calculated from data obtained on a minimum of 10,000 nuclei
[c] Immature leaves were sampled on adult coconut palms

immature leaves (80.3%). These data were confirmed by the percentage of cells in phases S and G_2/M—3.7% and 4.3%, respectively for the shoot meristems versus 11.5% and 8.4% for the leaves. This result might have been due to the fact that, unlike the shoot meristems, which were grown in vitro**,** the immature leaves used in this experiment were sampled from a palm cultivated in the field.

Effect of in vitro culturing of immature coconut leaves on the proportion of cells in the different phases of the cell cycle

In order to see whether in vitro culture interacts with the cell cycle in coconut tissues, we carried out an experiment assessing the distribution changes in cell-cycle phases before and after the inoculation of young leaves on the callogenesis medium.

The percentages of cells in different phases of the cell cycle before and at different times after they were placed in in vitro culture are shown in Table 2. Placing leaves in in vitro culture led to a significant increase in the percentage of cells in phase G_0/G_1—from 78.8% to 88% after 1 day of culture. At the same time, there was a drop in the proportion of cells in the S phase (falling from 12.5% to 3.3%), whereas the percentage of cells in the G_2/M phase remained stable (8.7–9%).

Between the first and fourth days of culture, the percentage of cells in phase G_0/G_1 fell from 88% to 84.4%; this decrease was accompanied by a significant increase in the percentage of cells in phase S (from 3.3% to 7.2%). The increase was only transient, since after 7 days of culture the percentage was 4.9%. This development coincided with the appearance of the first browning symptoms, generally described in coconut leaf tissues on the seventh day (Verdeil and Buffard-Morel 1995). Beyond the seventh day of culture, the percentage of cells in phase G_0/G_1 and in phase S stabilized around

Table 2 Effect of in vitro culturing of immature coconut leaves on the proportion of cells in the different phases of the cell cycle. Values are the means of five replicates. Results of one-way analysis of variance (ANOVA)are given: Snedecor's variable (F) and Type-I error (P) (*CIV* in vitro culture)

CIV duration (days)	Cell-cycle phases[a](%)[b]		
	G_0/G_1	S	G_2/M
0	78.8±3.5a	12.5±a	9±1.5a
1	88±1.2c	3.3±0.8c	8.7±0.7a
4	84.4±1.3b	7.2±1.2b	8.3±1.2a
7	87.1±1c	4.9±0.7c	8±0.7a
14	90.3±2c	4.1±0.9c	5.6±0.9b
28	89.6±2.2c	4±0.7c	6.4±1.2b
45	89.5±2.3c	4.6±1.3c	5.9±1.2b
60	88.6±2.1c	7±2b	4.3±0.9b
ANOVA—F	13.57119	15.81630	6.47095
ANOVA—P	0.000000	0.000000	0.000087

[a] Values followed by the same letter are not significantly different at the 5% level as determined by the Newman and Keuls' test
[b] Percentage calculated from data obtained on a minimum of 10,000 nuclei

89% and 4%, respectively The percentage of cells in phase G_2/M was then at its lowest level (approx. 4–6%).

Sixty days after the start of culture there was an increase in the percentage of cells in phase S (from 4% to 7%). The first divisions appeared in the perivascular tissues also at 60 days; the meristematic cells that will give rise to the calli are located in these tissues (Buffard Morel et al. 1992).

Testing of cell-cycle synchronization in phase S with aphidicholin on immature coconut leaves

The results given in Table 3 showed a significant increase in the percentage of cells in the S phase (from 4–6% to 20%) when the immature leaves were treated with aphidicholin (60 μ*M*) for 108 h. This increase was accompanied by a drop in the percentage of nuclei in phase G_0/G_1, which stabilized at round 68.3%. Seventy-two hours following the transfer of the immature leaves onto an aphidicholin-free medium, the percentage of cells in the S phase had recovered its initial value.

These data confirmed the possibility that a blocking of some of the coconut cells in phase S occurred with aphidicholin treatment. Aphidicholin is known to be an S-phase inhibitor in model plants (Mironov et al. 1999).

Discussion

To our knowledge, this is the first time that flow cytometry has been applied with success to coconut. Applying flow cytometry to coconut was a great challenge as the experiments were mainly done on heterogeneous tissues where a difference in the state of cellular differentiation—ranging from small, relatively uniform, densely cytoplasmic cells at the meristematic

Table 3 Effect of aphidicholin synchronization on immature coconut leaves cultured in vitro. Values are the means of five replicates. Results of one-way analysis of variance (ANOVA) are given: Snedecor's variable (F) and Type-I error (P)

Aphidicholin-concentrations (μ*M*)	Incubation times (h)	Cell-cycle phases[a](%)[b]		
		G_0/G_1	S	G_2/M
0	72	86.9±1.5b	4.5±0.3b	8.6±1.2a
0	108	83.5±3.2b	5.2±0.9b	11.3±1.5a
0	144	85.8±8.1b	6.4±1b	7.8±6.5a
30 μ*M*	72	90.9±1.7b	5.0±1b	4.1±0.8a
30 μ*M*	108	87.9±6.1b	8.0±1.2b	4.1±0.7a
30 μ*M*	144	79.4±7.1b	10.1±2.1b	10.5±2.7a
60 μ*M*	72	88.7±2.7b	6.0±1b	5.3±0.9a
60 μ*M*	108	68.3±1.4a	20.9±0.4a	10.8±1.2a
60 μ*M*	144	88.9±1.5b	7.4±1.3b	3.9±0.3b
90 μ*M*	72	88.3±1.3b	8.3±1.1b	3.4±0.3b
90 μ*M*	108	88.3±2.4b	6.8±1.3b	4.9±0.4a
90 μ*M*	144	89.5±1.8b	7.2±1.3b	3.2±0.6a
ANOVA—F		5.584439	6.799764	3.559099
ANOVA—P		0.000962	0.000263	0.011541

[a] Values followed by the same letter are not significantly different at the 5% level as determined by the Newman and Keuls' test
[b] Percentage calculated from data obtained on a minimum of 10,000 nuclei

regions to fully differentiated cells containing little or no cytoplasm—was found. This heterogeneity, which is characteristic of vascular plants, was demonstrated by Fox and Galbraith (1990) to be a major difficulty in assessing results from flow cytometry. To circumvent these difficulties, we tested and optimized each step of the method used (nuclei extraction, time of chopping, nuclei suspension filtration for limiting debris background, staining) for coconut tissues based on the recommendations of various authors (Dolezel et al. 1989; Noirot et al. 2000; Yanpaisan et al. 1999).

Flow cytometry allowed us to measure the nuclear DNA content (5.6±0.2 pg DNA/2C), and this is to our knowledge the first report for *Cocos nucifera* L. This value was found to be rather different from the one reported for other Arecaceae family species (Bharathan et al. 1994; Rival et al. 1997).

Flow cytometry applied to cell-cycle analysis in coconut palm tissues makes it possible to distinguish between tissues with a high potential for cell division and those with a low potential. This is shown by the fact that 25.7% of the nuclei from FGC were in phase S as opposed to 16.0% for the SGC. These results confirm those obtained in other species (tobacco, cotton and oat), for which it has been shown that the potential for cell division and regeneration was as high as the proportion of cells in phases S and G_2/M (Firoozabady 1986; Bergounioux et al. 1988; Chen et al. 1995).

All of the coconut tissues cultured in vitro displayed a high proportion of cells in the G_0/G_1 phase (around 90%). These experimental data need to be considered in relation to the slowness of the morphogenesis process that characterizes in vitro regeneration of the coconut palm: the first calli are obtained 3–4 months after the inoculation of primary explants on the callogenesis medium (Buffard-Morel et al. 1992; Verdeil et al. 1994).

Culture in vitro is known to reduce the rate of division in tissue (Winkelmann et al. 1998), and this has been described for coconut tissues (Jesty and Francis 1992), but the use of flow cytometry enabled us to specify that this phenomenon takes place in coconut within 24 h after the transfer from the in vivo to in vitro condition. Indeed, although no symptom was visible to the naked eye, flow cytometry revealed a rapid accumulation of nuclei in the G_0/G_1 phase in the leaves only 1 day after the start of culturing (88% of cells in phase G_0/G_1 1 day after in vitro inoculation of young leaves as opposed to 78.7% before culturing). This was probably linked to the stress caused by the culturing operation, which could induce in coconut a change in the relation between cell and nuclear size, leading thereby to an accumulation of cells in the G_0/G_1 phase (Jesty and Francis 1992). However, coconut cells seem to be able to temporarily overcome this stress, since 4 days after the start of culturing, there was a significant rise in the percentage of cells in the S and G_2/M phases (increasing from 84.3% to 87.1% and 7.2% to 4.8% in phases G_0/G_1 and S, respectively). This increase was only transient as 7 days after the start of culturing the percentage of cells in the G_0/G_1 phase stabilized at around 90%. This accumulation coincided with the appearance of the first browning symptoms due to the accumulation of polyphenols in the tissues. Polyphenols might have been one of the causes for the increase in the percentage of cells in the G_0/G_1 phase, and it would be worth studying their effects on the cell cycle by adding exogenous polyphenols, notably to FGC, which, compared to SGC, display a low susceptibility to browning and a higher ability to divide. Recent studies on polyphenols in green tea using animal systems revealed that they led to a blockage of cells in the G_0 phase (Kennedy et al. 2001).

The addition of aphidicholin to the medium significantly increased the percentage of cells in the S phase (increasing from 5.2% to 20.9%) despite the fact that this drug is known to be a specific DNA polymerase inhibitor that reversibly blocks G_1/S progression, resulting in an increase of cells in the G_1 phase (Ikegami et al. 1978; Mironov et al. 1999; Planchais et al. 2000). However, our

results are in accordance which what was observed in maize root meristems (Cuq et al. 1995) and, in some cases, in tobacco cell suspensions (Planchais et al. 2000). This can be explained by the heterogeneity of coconut tissue, which can perturb the action of aphidicholin that is known to act by slowing down (and not blocking promptly) replication (Planchais et al. 2000).

We calibrated the optimal concentration of aphidicholin (60 μ*M*), the duration of the treatment (108 h) and the time of re-entry to the cell cycle for coconut. Using these conditions (high concentration, long treatment duration), we were able to synchronize a proportion of cells, but the percentage remains low, unlike in model plants, where up to 80–90% of cells can be found in the S phase (Cuq et al. 1995; Planchais et al. 1997). The existence of a large percentage of cells in the G_0/G_1 phase (more than 90%) and a small percentage of cells in the S phase after the aphidicholin treatments prompts the hypothesis that a certain percentage of coconut cells cultured in vitro are blocked in the G_0/G_1 phase. Histocytological studies carried out on coconut have also revealed the frequent existence of nuclei with condensed chromatin that is typical of quiescent cells (Buffard-Morel et al. 1992; Verdeil et al. 1994). Studies at a molecular level are now being developed in order to understand the mechanisms controlling the switch from phase G_0 to phase G_1 and from G_1 to S in coconut cells.

This study on coconut revealed that a simple monoparametric flow cytometric method can be used to rapidly assess the ability of tissues cultured in vitro to divide. This is essential for coconut palm as this species is highly recalcitrant to regeneration and very slow to respond to in vitro treatment (Georges and Sherrington 1984; Verdeil and Buffard-Morel 1995). Flow cytometry will make possible a rapid observation of the effect of any given treatment and appears to be a useful tool for a more effective monitoring of the meristematic potential of tissues cultured in vitro (key point of in vitro vegetative propagation), as suggested by Yanpaisan et al. (1999). While we must keep in mind that a high rate of cell division does not necessarily lead to regeneration, the existence of a way to monitor the ability of cell division in coconut palm is an important step towards the mastering of regeneration for this particularly recalcitrant species.

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