In liquid medium colchicine treatment induces non chimerical doubled-diploids in a wide range of mono- and interspecific diploid banana clones

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Abstract — Introduction. The worldwide production of banana and plantain, which is estimated to 106 Mt·year^{-1}, relies on a narrow genetic basis. Thus, banana production is fragile regarding emergent diseases, and creation of new varieties of banana stands out as a real necessity for its sustainability. In banana, various improvement strategies aim to create triploid hybrids. The objective of this report is to present a simple *in vitro* methodology to induce stable tetraploid plants by colchicine treatment, usable as parent for obtaining triploid varieties. **Materials and methods**. Twenty-one diploid *M. acuminata* clones and three interspecific *M. acuminata / M. balbisiana* diploid clones were treated as proliferating culture in liquid medium with 1.25 mM of colchicine for 48 h. Plant screening has been performed by morphological identification in greenhouse, chromosome counts on root tips and flow cytometry on leave blades on vitroplants using propidium iodide staining. **Results**. Chromosome counts led to distinguish diploid and tetraploid plants but did not afford to detect chimeras. Flow cytometry allowed an early screening of a larger number of plants leading to detect rapidly chimerical plants. It was observed that some stable 2x / 4x cyto-chimeras are periclinal. Tetraploid clones were obtained with all diploid genotypes. General behaviour of tetraploids in the field was globally weaker than the corresponding diploids. Nevertheless, all the doubled-diploids flowered and crossed with diploid plants to obtain triploid progenies. **Conclusion**. This study has clearly shown that induction of stable doubled-diploid plants can be obtained from a wide range of genetically different bananas. These results open the way to the systematic use of doubled-diploids by banana breeding programs for the release of enhanced triploid varieties.

Guadeloupe / *Musa* / tissue culture / colchicine / chromosome number / tetraploidy / chimeras

La colchicine appliquée en milieu liquide induit des diploïdes doublés non chimériques dans une large gamme de clones de bananiers diploïdes mono ou interspécifiques.

Résumé — Introduction. La production mondiale de bananes et plantains, qui est estimée à 106 Mt·an^{-1}, s'appuie sur une base génétique étroite. La production de bananes est donc fragile quant à la menace des maladies émergentes et la création de nouvelles variétés apparaît comme une réelle nécessité pour sa pérennité. Les diverses stratégies d'amélioration du bananier visent à créer des hybrides triploïdes. L'objectif de cet article est de présenter une méthode aisée de production de plantes tétraploïdes stables par traitement de colchicine appliqué *in vitro*, utilisables comme parents pour obtenir de nouvelles variétés hybrides triploïdes. **Matériel et méthodes**. Vingt-et-un clones diploïdes de *M. acuminata* et trois clones diploïdes interspécifiques *M. acuminata / M. balbisiana* ont été traités durant la phase de prolifération *in vitro* dans un milieu liquide avec 1,25 mM de colchicine pendant 48 h. Le tri des plants a été effectué par une identification morphologique en serre, le comptage des chromosomes a été fait sur les pointes de racines et la cytométrie de flux sur fragments de feuilles de vitroplants en utilisant l'iodure de propidium comme colorant. **Résultats**. Le comptage des chromosomes a été efficace pour distinguer les plants diploïdes et tétraploïdes mais il n'a pas pu permettre de détecter les chimères. La cytométrie de flux a permis un criblage précoce d'un plus grand nombre de plants et a conduit à la détection rapide des plants chimériques. Il a été noté que quelques cytochimères 2x / 4x stables étaient périclinales. Des clones tétraploïdes ont été obtenus avec tous les génotypes diploïdes. En général, les tétraploïdes au champ se sont révélés moins vigoureux que les diploïdes correspondants. Néanmoins, tous les diploïdes doublés ont fleuri et ont été croisés avec des plants diploïdes pour obtenir des descendances triploïdes. **Conclusion**. Notre étude a clairement montré que l'induction de plants stables de diploïdes doublés peut être obtenue à partir d'une large gamme de bananiers génétiquement différents. Ces résultats ouvrent la voie à l'utilisation systématique de diploïdes doublés par les programmes d'amélioration pour l'obtention de variétés triploïdes améliorées de bananiers.

Guadeloupe / *Musa* / culture de tissu / colchicine / nombre chromosomique / tétraploïdie / chimère

1. Introduction

The worldwide production of banana and plantain is estimated to 106 Mt per year[1], of which 15 Mt are intended for the exportation markets and the remainder in food producing culture or for the supply to the local markets. The banana and plantain productions, which contribute to the basic nutrition closely of 400 M of individuals in the world, are subject to an increasing pressure of the diseases, of which the principal is the Black Leaf Streak Disease [1].

Most of the natural varieties are triploid, seedless and propagated by suckers. They are derived from the sole *Musa acuminata* species (AAA cv.) or from *M. acuminata* and *M. balbisiana* intercrosses (AAB cv. and ABB cv.). Diploid varieties (AA cv. and AB cv.) are less important [2] and natural tetraploids are very few. The current triploid varieties result from crosses between two diploid varieties in which one parent contributes a diploid gamete [2–4].

More than 45% of the world production relies on one narrow genetic subgroup, Cavendish, in which natural variability relies only on small mutation processes. Thus, banana production requires phytosanitary protections and is fragile regarding emergent diseases. Therefore, the creation of new varieties of banana stands out as a real necessity for production sustainability.

In banana, various improvement strategies aim to create triploid hybrids [5]. In diploids, Dodds and Simmonds [6] described how abnormal meiosis occurs in banana and induces diploid spores. Nevertheless, gamete restitution is fairly rare [7]. Thus, chromosome doubling applied to diploid clones is shown as an alternative pathway to systematize the obtention of triploids from crosses between diploids and doubled diploids.

Polyploidisation in banana was first induced by Vakili [8] by immersion of *M. balbisiana* germinating seeds in a colchicine solution. The recovered plantlets showed to be tetraploid and octoploid, some tetraploids reverting to diploid level. These first results on seeds were later confirmed by Dessauw [9] and Asif [10] on *M. balbisiana* and various *M. acuminata* subspecies and by Tesfaye [11] in the species *Ensete ventricosum*.

In 1992, Hamill *et al.* [12] reported first the induction of autotetraploid in the SH-3362 diploid edible hybrid. Polyploidisation resulted from the *in vitro* application of 1.25 mM (e.g., 0.5 w/v) colchicine for 2 h on micropropagated shoot tips in liquid medium. Selection of inducted tetraploids relied on gross morphology and stomatal characters. Stable autotetraploids have been established in the field, but reversions, formation of chimeras and aneuploids were common. Later, Van Duren *et al.* [13] compared, in similar *in vitro* conditions, the efficiency of colchicine and oryzalin to induce polyploidisation on the same SH-3362 clone. Most effective in producing non-chimerical tetraploids was 30 μM oryzalin for 7 days or 5 mM colchicine for 48 h. More recently, Ganga *et al.* [14] also reported the efficiency of colchicine in tetraploid induction of four mono- (AA) and interspecific (AB) Indian diploid banana cultivars. Clones were selected based on stomatal densities and chloroplast traits. Nevertheless, no mention was given on the stability of the regenerated tetraploids in the field.

The objective of our report is to present a simple *in vitro* methodology to induce stable tetraploids in bananas by colchicine treatment in a wide range of natural and synthetic edible diploid bananas. This methodology was used over a period of more than 15 years with a good efficiency. An efficient screening procedure by chromosome counts on root tips and flow cytometry is also presented.

2. Materials and methods

2.1. Plant material

All the diploid plant material used in our experiment was obtained from the *Centre de Cooperation Internationale en Recherche Agronomique pour le Développement* (CIRAD), Research Station of Neufchateau

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[1] http://faostat.fao.org/

Table I.
Characterization of 25 diploid *Musa* clones used to test the efficacy of colchicine treatment in order to induce doubled-diploids.

No. of the clone	Name of the clone	No. ITC[1]	Status	Genetic constitution	Cytoplasmic characterization[2]		Use	Origine
					Cp pattern	Mitotype		
1	AA cv. Rose	ITC 0712	Natural	AA	II	α	Dessert type	Indonesia
2	Colatina Ouro	ITC 0260	Natural	AA	II	α	Dessert type	Brazil
3	IDN 077	ITC 0688	Natural	AA	II	α	Dessert type	Indonesia
4	Gu Nin Chiao	ITC 0442	Natural	AA	II	α	Dessert type	Singapore
5	Khai Nai On	ITC 0663	Natural	AA	II	α	Dessert type	Thailand
6	Kirun (=Sucrier)	–	Natural	AA	II	α	Dessert type	Guadeloupe
7	Manang	ITC 0297	Natural	AA	II	α	Dessert type	Philippines
8	No. 110 / Tha 052	–	Natural	AA	II	α	Dessert type	Thailand
9	Pisang Lilin	ITC 0001	Natural	AA	II	δ	Dessert type	Malaysia
10	Pisang Madu	ITC 0258	Natural	AA	V	α	Dessert type	Malaysia
11	Pisang Pipit	ITC 0685	Natural	AA	II	α	Dessert type	Indonesia
12	Pisang Trimulin	ITC 0699	Natural	AA	III	α	Dessert type	Indonesia
13	Sa	ITC 0673	Natural	AA	II	α	Dessert type	Thailand
14	Tjau Lagada	ITC 0090	Natural	AA	III	α	Dessert type	Indonesia
15	Bebeck	ITC 0471	Natural	AA	V	α	Cooking type	Irian Jaya
16	Bie Yeng	ITC 0279	Natural	AA	V	α	Cooking type	Papua New Guinea
17	Galéo	ITC 0259	Natural	AA	V	α	Cooking type	Papua New Guinea
18	Guyod	ITC 0299	Natural	AA	II	α	Cooking type	Philippines
19	Gwanhour	ITC 0256	Natural	AA	V	φ	Cooking type	Papua New Guinea
20	Heva	ITC 0257	Natural	AA	V	α	Cooking type	Papua New Guinea
21	NBB11 / SF265	ITC 0270	Natural	AA	V	α	Cooking type	Papua New Guinea
22	Tomolo	ITC 1187	Natural	AA	V	α	Cooking type	Papua New Guinea
23	Col 49 – Guyod × Galéo	–	CIRAD hybrid	AA	V	α	Cooking type	Guadeloupe
24	Kunnan	ITC 1034	Natural	AB	II	δ	Dessert type	India
25	Gu 56 – BB × Galéo	–	CIRAD hybrid	BA	VIII	α	Cooking type	Guadeloupe

[1] ITC: International Transit Center code given by the International Network for the Improvement of Bananas and Plantains (INIBAP).

[2] According to the classification of the diploid varieties given by Carreel *et al.* [3].

in Guadeloupe (F.W.I.). These diploid clones were classified according to Carreel *et al.* [3]: twenty-one diploids are natural dessert or cooking *M. acuminata* clones. One natural interspecific AB clone (cv. Kunnan), one experimental interspecific BA cooking type hybrid and one experimental AA cooking type hybrid were also experimented (*table I*). These clones are representative of the wide genotypic and phenotypic diversity of the edible diploid bananas around the world [2].

2.2. Methods

2.2.1. Establishment of the proliferating culture

Tissue cultures were initiated from shoot tips taken from banana suckers. For sterilisation, banana suckers were reduced to explants of 2–3 cm in length and 2 cm in diameter; they were then immersed in 95% (v/v) ethanol, followed by flaming. Flaming was repeated two times. Burned tissues

were then discarded, in sterile conditions, up to innermost intact tissues. Shoot apices were placed onto 100 mL of a solid proliferating medium containing MS macro- and microelements [15], vitamins from Morel [16], 88 mM sucrose, 22.2 µM 6-benzylaminopurine (BAP), 651.5 µM adenine sulphate and 7 g·L^{-1} agar. The pH of the medium was adjusted to 5.8 before autoclaving. Cultures were kept at 27 °C in darkness for 45 days. Active multiplication occurred following subdivision of bud clusters and subculture of individual pieces on proliferating medium in the same conditions for more than 45 days. At this stage, the explants were divided to sub-explants of 2-cm diameter and individually transferred, for 10 days, to Erlenmeyer flasks containing 125 mL of proliferating liquid medium of same composition but complemented with 3mM 2-[N-morpholino] ethanesulfonic acid (MES) and without agar. All the cultures in liquid medium were performed on a gyratory shaker (120 rpm) in a growth chamber at 27 °C and 16 / 8 h (day / night) photoperiod (Mazda "daylight" fluorescent tubes, 100 µmol·m^{-2}s^{-1}).

2.2.2. Colchicine treatment and plant recovery

The proliferating clusters were subdivided again and individually subcultured in 125 mL of the proliferating liquid medium supplemented with 1.25 mM colchicine (dissolved in 90% ethanol) for 48 h. Explants were then taken out of the flasks, placed in 125 mL of sterile distilled water for 2 days and transferred in the proliferating liquid medium for 15 days. The clusters were then replaced on the proliferating solid medium until bud proliferation. Buds were isolated from the cultures and subcultured onto the hormone-free solid medium (without BAP and adenine sulfate) to allow growth of the shoots.

Grown microshoots were isolated from the clusters and then placed individually in tubes on the hormone-free solid medium to induce formation of elongated shoots and roots. The plantlets were transferred 30 to 40 days later to nursery on an artificial substrate (Giffy-7, Giffy Products Ltd., Norway), at a temperature of 25–26 °C at night and 30–35 °C during the day, under natural light

of 40–150 µmol·m^{-2}·s^{-1} and 75–100% relative humidity. Thirty days later, plants were finally transferred to 3-L pots with a mixture of river sand and peat, enriched with 10 g per plant of complete fertilizer (N-P-K and microelements), and put in greenhouse.

2.2.3. Plant characterization

2.2.3.1. Morphological identification

In greenhouse, 45-days-old tetraploid plants were usually more robust stocky and had thicker leave blades than diploids. Tetraploids also showed a higher resistance to rolling up of leave blades between fingers than diploids. All these indications were used to early identification, in greenhouse, of putative tetraploids pre-selected for more accurate characterization.

2.2.3.2. Chromosome counts

Chromosome counts were carried out according to Bakry and Horry [17]. The root tips were taken from the plants in the greenhouse between 6:30 A.M. and 8:00 A.M. and placed in 0.03% (w/v) hydroxyquinoline solution, open to the air at 20–25 °C for 6–8 h. The tips were then immersed for at least 3 h into a macerating/fixing mixture (acetic acid:ethanol:water, 4:1:5). Finally, the root tips were crushed between the microscope slide and cover-slip in a drop of stain [orcein 2% (w/v) in a solution of lactic acid:phenol:glycerol:water, 1:1:1:1]. Counting was carried out at 1000 (10 × 100) magnification. For each plant, chromosome counts were performed on five to six independent cells from one or two root tips.

2.2.3.3. Flow cytometry

For the flow cytometry studies, samples of 1 cm^2 of young plant leaves taken off in the greenhouse were chopped with a razor blade in 55-mm-diameter Petri dishes, containing 0.5 mL of Phosphate Buffered Saline (PBS) solution as extraction buffer, completed with Triton X-100 (0.25%) and sodium meta-bisulfite (1%). The suspension of released nuclei was passed through a 15-µm Nylon filter and mixed with 0.2 mL of extraction buffer supplemented with propidium iodide (200 µg·mL), which is a DNA

intercalating dye [18]. Before analysis, preparations were maintained for 5 min in the dark and on ice. Four samples were prepared for each of the clones. Measurements were performed on a FACScalibur Laser flow cytometer (Beckton Dickinson, USA). Histograms of FL2 fluorescence intensity were registered over 1024 channels and studied using WinMDI$^{©}$ software Ver. 2.8.

Measurement of the size of the banana genome was performed with the internal control *Citrus* limon 'Lisbon', whose absolute genome size had been previously measured at 0.786 pg / 2C [19, 20]. Nuclear 2C DNA content was estimated for each diploid clone with the formula [(average channel number of banana nuclear peak / average channel number of *Citrus* limon cv. Lisbon peak) × 0.786 pg].

2.2.3.4. Histology

Histological studies were performed on *in vitro* banana shoot apex of Americani cv. (Cavendish, AAA) for comparison. Slides were prepared according to Bakry and Rossignol [21].

3. Results and discussion

3.1. Chromosome counts

For chromosome counts, chromosome should be well separated showing few overlappings so that chromosomes can be unequivocally defined and counted (*figure 1*).

Chromosome counts were performed in 88 different cells. Root tips taken from the same treated derived plantlets always showed the same chromosome number, either 22 chromosomes in the untreated donor plant, or 44 chromosomes in the colchicine treated ones. No diploid/tetraploid chimera was identified within the same root tip and no plant with higher chromosome content was observed.

3.2. Flow cytometry

Flow cytometry used for early ploidy screening led to quantify the DNA content of the
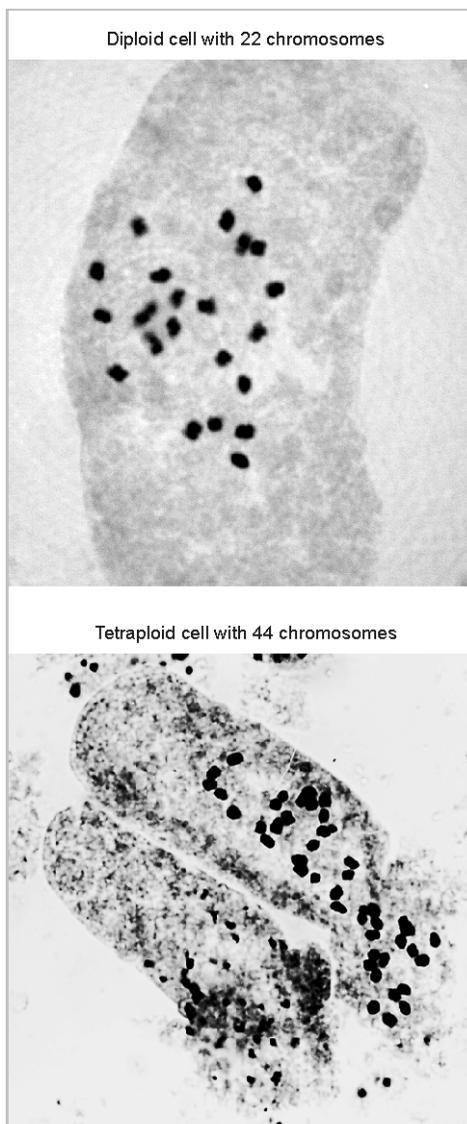

Figure 1.
Chromosome counts: plates showing a diploid cell containing 22 well-separated chromosomes and a tetraploid cell containing 44 chromosomes with some superpositions.

25 diploid varieties submitted to colchicine treatment. Average DNA content of the AA clones was 1.13 pg per nucleus, ranging from 1.07 pg per nucleus for Pisang Lilin to 1.17 pg per nucleus for Bebeck (*table II*). Kunnan (AB) showed a low DNA content, which is consistent with its interspecific genomic composition, *M. balbisiana* owning one of the smallest DNA content in the *Eumusa* section (unpublished results). These results are consistent with those of previous reports on *Musa* [22].

Table II.
Ploidy status of 25 *Musa* clones after in liquid medium colchicine treatments, determined by the use of chromosome counts and flow cytometry.

No. of the clone	Name of the clone	Year of analysis		Number of plants analysed by:						DNA content pg/2C
				Chromosome counts[1]		Flow cytometry[2]				
		Chromosome counts	Flow cytometry	2N = 22	2N = 44	2N = 22	2N = 44	Chimeras 2N = 22/44	Other chimeras	
1	AA cv Rose	1988	1993	2	8	0	0	20**	0	1.11
2	Colatina Ouro	–	1995	–	–	0*	2*	4*	0	1.15
3	IDN 077	–	2002	–	–	3**	6**	8**	0	1.10
4	Gu Nin Chiao	–	1995	–	–	1*	2*	4*	0	1.14
5	Khai Nai On	–	1994	–	–	0*	8*	0*	0	1.15
6	Kirun (=Sucrier)	–	1998	–	–	4*	18*	13*	0	1.13
7	Manang	–	1995	–	–	2*	4*	14*	0	1.12
8	N°110 / Tha 052	–	2004	–	–	4**	4**	2**	0	1.13
9	Pisang Lilin	1988	1993	2	2	0	5**	0	0	1.07
10	Pisang Madu	–	2002	–	–	2**	8**	6**	0	1.11
11	Pisang Pipit	–	1994	–	–	0	3**	0	0	1.15
12	Pisang Trimulin	–	1998	–	–	16*	17*	33*	0	1.15
13	Sa	–	1994	–	–	0	11**	0	0	1.15
14	Tjau Lagada	1988	1994	2	13	2*	3*	4*	0	1.14
15	Bebeck	–	1995	–	2	0	3**	5**	0	1.17
16	Bie Yeng	1988	1993	4	3	0	–	14**	0	1.14
17	Galéo	1988	1993	1	3	0	2**	7**	0	1.13
18	Guyod	1988	1993	6	11	0	9**	1**	0	1.16
19	Gwanhour	–	1995	–	–	4*	5*	9*	0	1.10
20	Heva	–	1994	–	–	2*	12*	1*	0	1.14
21	NBB11 / SF265	1988	1993	2	5	0	3**	0	0	1.14
22	Tomolo	–	1995	–	–	6*	7*	2*	1:4*x* / 8*x**	1.11
23	Col 49 – Guyod × Galéo	–	1998	–	–	2*	21*	19*	6: 4*x* / 8*x*	–
24	Kunnan	–	2004	–	–	0**	23**	6**	0	1.07
25	Gu 56 – BB × Galéo	–	1995	–	–	0	4**	1**	0	–

[1] Chromosome counting on roots tips on plantlets in the greenhouse.

[2] Flow cytometry measurements on *in vitro* plantlets (*) or on plantlets in the greenhouse and/or on plants in the field (**).

Three different situations were observed for ploidy screening (*figure 2*):

– histograms with two close peaks, the citrus control on the left and the diploid *Musa* peak on the right;

– histograms with two distant peaks, the citrus control on the left and the *Musa* tetraploid peak on the right;

– histograms with three peaks, the citrus on the left and two *Musa* peaks on the right corresponding to the diploid and tetraploid tissues of the chimeras.

During the whole study, more than 400 individual plants were screened by flow cytometry (*table II*). The quantity of plant material analysed by flow cytometry is much higher than by chromosome counts. Moreover, thanks to flow cytometry, cytochimeras directly identified at *in vitro* level were discarded before weaning in greenhouse. Furthermore, flow cytometry allowed a

better selection since some clones (AA cv. Rose and Bie Yeng) evaluated as tetraploid by chromosome counts were revealed to be stable diplo-tetraploid chimeras after several years in the field. With this example, the lack of non-chimerical plants for these both varieties is analysed as a consequence of a selection of plants with 44 chromosomes in greenhouse and the capability of these 2x / 4x cytochimerical plants to produce gametes with 22 chromosomes in the field.

Flow cytometry is less time consuming than classical cytology and fits well for early mass screening of the polyploid plantlets. However, it should be emphasized that chromosome counting remains a simple and cheap method for ploidy characterization; it has been used advantageously before the availability of flow cytometry.

3.3. Production of stable tetraploid clones

Results (*table II*) show that *in vitro* liquid colchicine treatment succeeded in the tetraploidization over a wide range of diploid clones and gave rise to 23 non-chimerical autotetraploid clones. Chromosome-doubling has been obtained on varieties of pure *acuminata* genomic constitution as on varieties of interspecific *acuminata / balbisiana* constitution. Because meristematic tissues are easily and rapidly in contact with medium, liquid medium looks more suitable than semi-solid medium for the colchicine treatment phase as for the post treatment reactivation and regeneration of the doubled tissues. All the tetraploid plants were transferred to the field and they remained stable after many years of observation. No reversion to diploid level has ever been observed until today. This stability in the field might be considered as the result of an accurate *in vitro* selection of the plant material by flow cytometry.

A high proportion of the material regenerated after colchicine treatment proved to be chimerical with respect to its ploidy level (20 varieties). Chimerical plants have often been obtained by various authors with banana [8, 12–14]. The production of "mixoploid" plants clearly supports the hypoth-
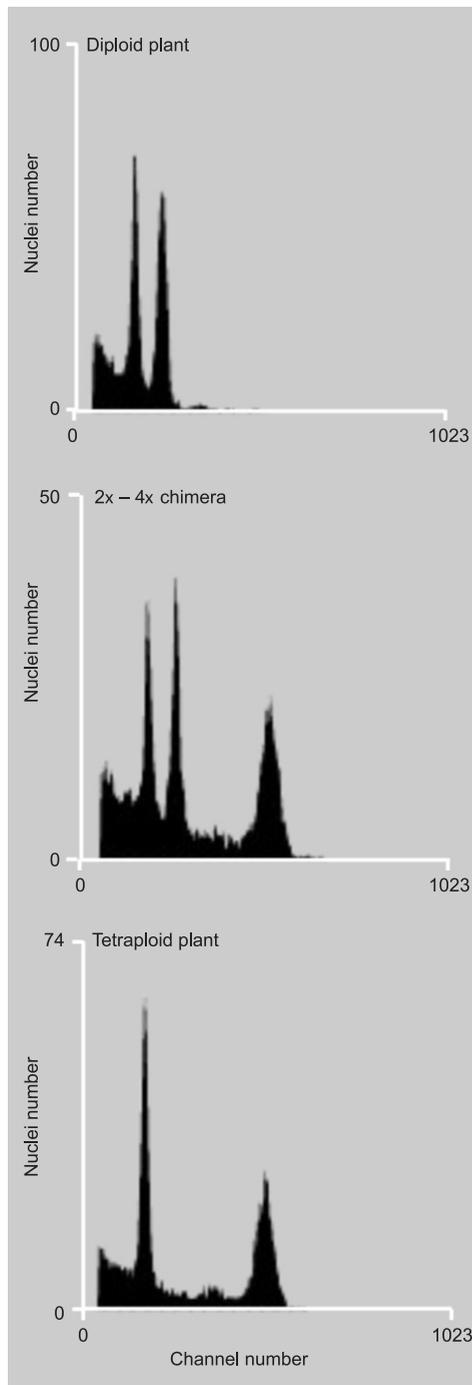

Figure 2.
Histograms of relative nuclear DNA content obtained after flow cytometry using propidium iodide staining. On each histogram, the *Citrus* lemon peak which serves as internal reference is on the left, and the *Musa* peaks are on the right: *M. acuminata* peak of a diploid clone (2N = 2x = 22), two *M. acuminata* peaks corresponding to 2x / 4x chimerical clones, *M. acuminata* peak of a doubled–diploid clone (2N = 4x = 44).

esis of a pluri-cellular origin of the *in vitro* induction of shoot meristems in banana.

The origin and the constitution of the stable cytochimeras observed in the field remain

Figure 3.
Median longitudinal section through the shoot apex of a vitroplant of cv. Americani (Cavendish, *Musa acuminata*): leaf primordia encircling the central primordia dome.
In vitro, anticlinal and periclinal cell divisions occur just below the epidermic L1 layer.
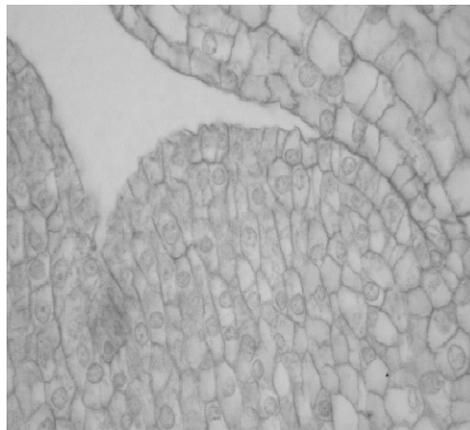
unclear. These plants are producing gamete containing 22 chromosomes whereas flow cytometry results clearly confirm their mixoploid constitution at leaf level. In banana, our own observations on *in vitro* grown vegetative apex show that cells below tunica are multiplying actively (*figure 3*; unpublished results). Thus, these cell layers are supposed to be more susceptible to the colchicine treatment than those in the more internal regions.

Barker and Steward [23] observed that cell divisions in the central area of the banana vegetative meristem are sparse *in vivo*, while the main organ-building and cell-multiplying functions are delegated to the lateral organs. On another hand, Fahn *et al.* [24] recognized five cytohistological zones in the banana vegetative shoot with a tunica at the summit of the apex. Some anticlinal and periclinal cell divisions occur in second tunica layer and in central-mother-cell zone, but more mitotic activities occur at the periphery of the apex. In *Musa*, it is only during the transition from the vegetative to the floral phase of the shoot that the cells in central position below tunica enter into high mitotic activity. All these observations are consistent with the hypothesis that these stable leaf mixoploid chimeras are probably periclinal and originated in the shoot apex from tetraploid tissues located in the central mother zone and diploid tissues located below [25, 26]. Whatever be the reality, these mixoploid plants have proved to constitute a reliable plant material to study the ontogeny of the vegetative meristem in banana.

3.4. Plant behaviour in the field

All the doubled-diploid plants were transferred to the field and observed during several vegetative cycles. Compared to diploid plants, tetraploid ones often bear drooping leaves; they also display a global reduction of vigour and a higher inhibition of suckering. This inhibition has no consequence for varieties presenting high suckering at the diploid level (e.g., Pisang Lilin, AA Rose cv., Khai Nai On, etc.). But, it showed to be more problematic for the cooking types from Papua New Guinea (e.g., Bebeck, Bie Yeng, NBB11/SF265) since these clones, which usually manifest a high inhibition of suckering even at the diploid level, do not exhibit any suckers at the tetraploid level. *In vitro* culture of inflorescences [27] was thus necessary to recover new plantlets for the propagation of plant material.

The reduction of vigour observed in the tetraploid material in comparison to diploid plants has been recorded on all accuminata genotypes. This behaviour has been already reported by others authors in pineapple [28] and in *Musaceae* [11]. It could be linked to a global reduction of heterosis.

All the doubled-diploids flowered in the tropical conditions of Guadeloupe. These plants were used as parents in crosses with diploid clones, which resulted in the production of triploids indicating that the gametophytes derived from these plants were diploid.

4. Conclusion

This study has clearly shown that induction of stable doubled-diploid plants can be achieved in a wide range of genetically different bananas. Chromosome counts cannot help to detect chimeras but may be useful for a rough selection of polyploid plants when flow cytometry is unavailable. Flow cytometry has shown to be efficient for *in vitro* detection of chimeras and the early selection of tetraploids. Moreover, this technique allows working easily on a large number of plants.

These induced tetraploid plants have been established in the field and developed normally. The vigour decrease observed in

the field in the doubled-diploid accuminata plants is not a constraint since their interest does not rely on their own phenotypic value but on their ability to transmit the phenotypic value of the original diploid clone to triploid progenies [29].

In our study, all plants have flowered bearing often true-to-type inflorescences with well-conformed female and male flowers. Their ability to produce diploid gametes has been verified over several cycles throughout the production of triploid progenies in crosses with diploids. Since the obtaining of 2N gametes is rare and uncontrolled in banana, the implementation of *in vitro* chromosome doubling allows and systematizes the synthesis of triploid hybrids directly from a wide range of diploid germplasm. Henceforth, this pathway will be effectively used by our breeding program for the synthesis of enhanced triploid varieties.

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La colchicina aplicada en medio líquido induce diploides doblados no quiméricos, en una amplia gama de clones de plataneros diploides mono o interespecíficos.

Resumen — Introducción. La producción mundial de plátanos y llantenes, que se estima a Mt·año^{-1}, se basa en una estrecha base genética. Por lo tanto la producción de plátanos es débil respecto a las enfermedades emergentes, y la creación de variedades nuevas aparece como una necesidad real para su perennidad. Las diversas estrategias de mejora del platanero pretenden crear híbridos triploides. El objetivo de este artículo es presentar un método fácil de producción de plantas tetraploides estables mediante tratamiento de colchicina aplicada *in vitro*, de modo a que se utilicen como progenitor con el fin de obtener nuevas variedades híbridas triploides. **Material y métodos**. Se trataron durante la fase de proliferación *in vitro* en un medio líquido con 1.25 mM de colchicina durante 48 h, veintiún clones diploidos de *M. acuminata* así como tres clones diploidos interespecíficos *M. acuminata* / *M balbisiana*. La clasificación de los plantones se efectuó mediante una identificación morfológica en invernadero, el recuento de los cromosomas se llevó a cabo en las puntas de las raíces, y la citometría de flujo se hizo sobre las hojas de vitroplantones utilizando para ello yoduro de propidium como colorante. **Resultados**. El recuento de los cromosomas fue eficaz para distinguir los plantones diploidos y tetraploidos, pero no logró detectar las quimeras. La citometría de flujo permitió un cribado precoz de un número de plantones mayor, y condujo a la rápida detección de plantones quiméricos. Se tomó nota que ciertas citoquimeras $2x$ / $4x$ estables eran periclinales. Se obtuvieron clones tetraploidos con la totalidad de los genotipos diploidos. De modo general, los tetraploides en campo labrado resultaron ser menos vigorosos que los diploides correspondientes. No obstante, todos los diploides doblados florecieron y se cruzaron con plantones diploides para obtener descendencias triploides. **Conclusión**. Nuestro estudio mostró claramente que la inducción de plantones estables de diploidos doblados puede obtenerse a partir de una amplia gama de plataneros genéticamente diferentes. Los resultados en cuestión abren la vía a la utilización sistemática de diploidos doblados para los programas de mejora para la obtención de variedades triploides mejoradas de plataneros.

Guadalupe / *Musa* / cultivo de tejidos / colchicina / número de cromosomas / tetraploidia / quimera