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Full Length Research Paper

Comparative study of *in vitro* regeneration efficiency of shoot-tip explants of *Pelargonium graveolens* and some cultivars of banana (*Musa* spp.) grown in Rwanda in response to different concentrations of cytokinins

UGIRINSHUTI Viateur[1]* DHED' A DJAILO Bénoît[2] and KABERA Justin Nzeyumwami[1]

[1]Institute of Scientific and Technological Research (IRST), Biotechnology Unit, Ruhengeri City, Rwanda.
[2]Kisangani University, Yangambi Campus, Faculty of Agronomy, Kisangani City, D.R Congo.

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This study examined the *in vitro* proliferation efficiency of shoot-tip explants from four cultivars of banana (*Musa spp*) and one species of *Pelargonium graveolens*. The explants were cultured on Murashige and Skoog (MS) medium supplemented with growth regulators. The comparative *in vitro* proliferation efficiency of banana cultivars was in the following order: Bluggoe > Injagi > FHIA-25 > Kamaramasenge, the comparative ratio was 10:7:4:2. The best number of shoot buds proliferation was observed on MS medium supplemented with 100 µM BAP while the quick buds development was observed on culture media with 10 µM BAP. The ANOVA proved that the genotype of explants of banana, the concentration in BAP and their interaction have high significant influence on their *in vitro* proliferation rate. Bluggoe, Injagi and FHIA-25 were described as subjects favorable for Rwanda's *in vitro* propagation program of high-performance plant varieties. *P. graveolens* required high quantity of hormones and bit complicated disinfection compared to banana. For *P. graveolens*, the medium MS + 100 mM BAP + 1 mM NAA + 1 mM GA_3 was the best for *in vitro* regeneration while the addition of 10 and 100 mM Kin favored the rooting. The results of this study contributed a lot to the *in vitro* regeneration, with minimal explants material of banana (*Musa* spp.) *a*nd *P. graveolens* currently carried out in Rwanda by ISAR and IRS, respectively.

Key words: Banana cultivars, growth regulators, *in vitro* proliferation, MS medium*, Pelargonium graveolens*, ratio, cytokinin/auxin, Rwanda, shoot-tip explants.

INTRODUCTION

In Rwanda as elsewhere in the world, banana (*Musa* spp.) is among the largest food crops and affects lives of more than 400 million persons and Rwanda appears among the big African countries producers of bananas. According to previous surveys, Rwanda occupied the second place on the list of the world consumers of banana (197 kg/person/year) after Uganda and the average consumption was 250 kg/person/year (Nasir and Titov, 2007; Frison et al., 1999; MINECOFIN, 2002) which indicated that it was higher than the average consumptions' of other places in the world. In Rwanda like elsewhere in the world, the production of banana was impeded by many factors, biotic as well as abiotic, and consequently, the bananas produced in Rwanda do not

*Corresponding author. E-mail: ugiraviateur@yahoo.fr. Tel: 00250788548358. Fax: + (250)252530939.

Abbreviations: ANOVA, Analysis of variance; **BAP,** 6-benzylaminopurine; **CM,** culture medium; **IAA,** indole-3-acetic acid; **GA_3,** gibberellic acid; **IITA,** international institute of tropical agriculture; **Kin,** kinetin; **MINECOFIN,** ministry of finance; **NAA,** naphthalene acetic acid; **MS,** Murashige and skoog.

satisfy the food and industrial need of Rwandans which grows day after day (Ortiz et al., 2002, Tripathi et al., 2009). The development of the dangerous diseases that have been the major consequence of the bad yield of most of banana varieties and the need to improve genetically the banana directed more and more efforts towards the alternative ways using the biotechnological techniques including the *in vitro* culture and regeneration of the entire plants from plant tissues (Cronauer, 1994; Dhed'a et al., 1991).

To increase the *in vitro* regeneration rate and to obtain the plants with good sanitary quality, *the in vitro* multiplication starting from various plant parts including shoot-tips was developed and is now being used in several countries around the world (Auboiron et al., 1998). The development of the new technique of regenerating the whole plant from embryogenic cell suspensions in banana has revolutionized the work of massive multiplication and genetic quality improvement of this plant (Strosse et al., 2006). Nowadays, they constitute the material of choice for rapid multiplication, cryoconservation (Bui and Tran, 2000; Panis et al., 2007) and genetic manipulation of most of banana species to provide them with resistance to various diseases and other beneficial traits (Kalim et al., 2009).

Accordingly, the technique of *in vitro* multiplication of the plants bearing the socio-economic interest was recently adopted by the Rwandan government and today it is jointly applied by various local research institutions among others, the Institute of Scientific and Technological Research (IRST), Rwanda Agricultural Research Institute (ISAR), and some universities. Nevertheless, at the day of the introduction of this technique by our country, there was not deep knowledge about the protocols for the successful micropropagation of the majority of the plants concerned by this technique as a matter of priority because they were not locally subjected to the *in vitro* culture yet.

Being motivated by the good initiative of our government in general and our research institution in particular, and the role played by the banana and the geranium (*P. graveolens*) in the everyday life Rwandans, we ran this study aiming at evaluating the efficiency of these two plants in proliferating *in vitro* and the identification of the culture media favoring their best *in vitro* regeneration. In this study, the plants species were not chosen at random. The cultivars of banana Bluggoe (locally named Kivuvu), Injagi, FHIA-25 and Kamaramasenge (known as Ney Poovan) were selected, thanks to their agronomical and/or economic importance. Kamaramasenge (diploid AB) was chosen, thanks to the sweetness and good color of its fruits which confers to this cultivar an excellent quality and a high national and international market. Injagi (triploid AAA) was chosen, thanks to its tolerance to several kinds of grounds, its particular taste and the weight of its bunch of about40 kg. (Patrick Karangwa, Rwanda Agricultural Research Institute, personal communication). Bluggoe (triploid ABB), locally called Kivuvu, was studied, thanks to the weight of its bunch and the preference by the consumers (Dhed'a et al., 1991). FHIA-25 (tetraploid AAAB) was newly introduced in Rwanda and appeared to express a great economic interest. A mature bunch of FHIA-25 can weigh up to 80 kg. In addition, the brewing of its fruits revealed itself to be very easy and fast compared with other varieties (Ferreira et al., 2002; Ploetz et al., 2007). Due to the size and the robustness of its pseudo-trunk, FHIA-25 is resistant to strong winds and other mechanical aggressors. As for the *P. graveolens*, the choice was based on its pharmaceutical and other properties.

Few years ago, the IRST has run various assessments on its potentials in fighting the mosquitoes responsible for malaria and most of plant pathogens in Rwanda. The results of the assessments already done were very promising and showed that this plant exhibits both the biocidal effects on many of phytopathogenic fungi and the repellent effect on most of mosquitoes, fleas and other flying insects (Kabera et al., 2011; Botha and McCrindle, 2000). The aim of this study was to determine the capability of *in vitro* proliferation of the aforementioned banana cultivars and *P. graveolens* on different culture media and subsequently find out the factors that influence their potentiality of shoot buds formation. It contributes directly or indirectly to the establishment of a platform in the diffusion of banana varieties in our country and even in East Africa region. It aims at the sustainable, stable and high production of banana which are locally and internationally preferred or appreciated by the consumers, the food safety and the increase of income generated by the population through a strategy of production at low cost with respect to the environment.

MATERIALS AND METHODS

The young shoots of four cultivars of banana were taken from the banana germoplasm collection of ISAR at Rubona in Southern province of Rwanda.

This collection includes several varieties of banana trees representing three ploidy levels (di-, tri- and tetraploidy). The names of the four chosen cultivars of banana, their characteristics and their use were reported by the farmers and some authors (Carrel et al., 1994; Ferreira et al., 2002; Nsabimana et al., 2008; De Langhe, 2000). These are Kamaramasenge (AB and cooking and wine variety), Injagi (AAA and cooking variety), Bluggoe (ABB and cooking variety and FHIA-25 (AAAB and wine variety).

The representation of every ploidy level and the exhibition of higher agronomic and/or economical interest were the two main selection criteria. As for *P. graveolens* (geranium roast), the shoot-tip explants were extracted from young plants in the collection of medicinal and essential oils plants of IRST at Kinigi in Northern Province of Rwanda. The *in vitro* cultures were carried out in the in the laboratory of plant tissue culture of ISAR in Ruhengeri and Rubona.

Preparation of the culture media for banana cultivars

Stock-solutions

The preparation of the stock-solutions enabled us to avoid the everyday manipulations, and facilitated the preparation of the day-solutions which usually uses small quantities that are difficult to weigh. The composition of stock-solutions was made of the macro-elements (chemicals in big amount), microelements (chemicals in small amount), antioxidants, vitamins and growth regulators (Murashige and skoog.1962)). The composition of this culture media is given in the table 1 below.

Day-solutions

Four types of culture media with different concentrations in BAP (0, 1, 10 and 100 µM) were prepared (Sandeep et al., 2010). The preparation of these culture media followed the protocol established in the Laboratory for Tropical Crops Improvement at Katholieke Universiteit Leuven (KULeuven), Belgium, and already in use by the International Institute of Tropical Agriculture (IITA). However, some of its points were modified due to some circumstances and the following stages were followed without inverting them (Banerjee and De Langhe, 1985; Vuylsteke, 1998). In a flask of 1 L, 500 ml of distilled and sterile water was put and 30 g of saccharose was added and dissolved. Various quantities were taken from beforehand prepared stock-solutions and added to the mixture as showed in the Table 2.

The mixture was poured into a graduated cylinder and adjusted to 990 ml. The pH of the mixture was adjusted to 5.8 with 1N NaOH and/or 1N HCl (Sandeep et al., 2010) The mixture was then adjusted to 1l and was brought back in an Erlenmeyer in which 8 g of agar were added and dissolved by heating until boiling. Using a dispenser, the liquid and hot culture medium was distributed in the test tubes at the rate of 20 ml per tube, autoclaved at 121°C for 20 min, and finally cooled to solidify.

Preparation of explants of banana and inoculation of culture media

The preparation of explants consisted in surface sterilization of three steps, the dissection, disinfection and excision of the harvested shoots until the obtaining of internal part called the meristematic apex. The sword-shoots were taken from their mother-plants and the roots and leaves were removed. The explants were washed with the running tap water and cut with a knife in order to remain only with cubic explants of approximately 3 cm^3 each one. The explants obtained were plunged in the ethanol solution 70% (v/v) for 20 s, and then in the sodium hypochlorite solution 3% (w/v) mixed with Tween 20 (at the rate of 5 drop/l of sodium hypochlorite) for 15 min. The disinfected explants were rinsed 3 times in distilled and sterile water. Under sterile laminar and by using pincers for dissection and a scalpel armed with a lancet all sterile, the foliar sheaths of explants were delicately removed one by one as shown by the figure 1 until an apex of about 1 cm^3 of volume covered by 3 to 6 white and immature foliar primordia supported by a small corm was obtained. The explants were isolated by straight incisions at the level of the corm and at few millimeters below the junction of the apex and the corm. The obtained conical explants were put on the solidified and sterile culture media in such a way that the bases of the explants are in direct contact with the medium and that the explants are placed vertically straight. The cultures were then incubated in growth chamber at 27 ± 2°C, under the photoperiodicity of 16/18 (light/darkness) with the luminosity of 6000 lux from cool fluorescent tubes.

Preparation of culture media for *P. graveolens*

For the investigation of response of *P. graveolens* to *in vitro* plant regeneration, five types of culture media were prepared with a pH of 5.9 adjusted with 1 N NaOH and/or 1 N HCl. They were totally different from the media culture used for banana *in vitro* regeneration. The Murashige and Skoog medium was used as the basic ingredient and the concentration and types of cytokinins were different (Murashige and skoog, 1962). The protocol's steps of the preparation of these culture media were the same as those of the protocol used for banana *in vitro* culture with different quantities and types of hormones as an exception. The hormones and respective quantities are shown in table 3 below.

Preparation of explants of *P. graveolens* and inoculation of culture media

The explants were isolated from young plants of *P. graveolens* and their leaves were directly removed. The explants were washed with tap water and were put to drain under the air. Some of these explants were soaked in ethanol solution 70% (v/v), and others were pulverized with ethanol of the same concentration. A small number of these explants was treated separately with the sodium hypochlorite solution 3.5% (w/v) mixed with Tween 80 at the rate of 5 drops/ liter, others in the mercury chloride solution 2% (w/v) and others in the calcium hypochlorite solution 5% (w/v) for 15 min. All the explants were rinsed consecutively in separate three batches of distilled and sterile water for 5, 10 and 5 min, respectively and put to drain. Aseptically, the peduncles of explants were delicately removed with sterile scalpel equipped with lancet and the explants were cut into small pieces and put on the culture media supplemented various growth regulators and contained in the small punnets. The cultures were then incubated in the growth chamber at 20 ± 1°C, under the photoperiodicity of 16/18 (light/darkness) with the luminosity of 3000 lux from cool fluorescent tubes.

Statistical analysis

This analysis was made only for the banana in order to identify the factors that influence the capacity of *in vitro* proliferation of the 4 cultivars studied to cause the variability of their *in vitro* proliferation rate. To facilitate this analysis, we made available 4 explants by cultivar and by type of culture medium, each one constitutes a repetition, and the experiment was done in triplicate. In this study, the majority of the growth conditions offered to all explants were the same except the genotypes of explants and the concentration of the culture media in BAP. This situation led us to formulate the alternative hypothesis saying that these two parameters and/or their interaction, separately or in together, influenced the rate of *in vitro* proliferation of these four cultivars of banana. Therefore, we used the ANOVA II that uses cross statistical models (2 parameters and their interaction) to identify the true source of variability of the *in vitro* proliferation potentialities of four cultivars. The calculations were done under a risk α= 5% or 0.05 and α =1% or 0.01 (probability p = 0.95 and 0.99).

RESULTS

Response of four cultivars of banana to the different concentration of BAP

Along this investigation, the evolution of the banana

Table 1. Chemical composition of stock-solutions used to prepare the culture medium for *in vitro* regeneration of the four banana cultivars.

Concentration (g/L)	Component	
	Chemical formulas	**Stock-solutions**
38	KNO_3	
33	NH_4NO_3	Stock-solution A (Macroelements I)
8.8	$CaCl_2.2H_2O$	
7.4	$MgSO_4.7H_2O$	Stock-solution B (Macroelements II)
3.4	KH_2PO_4	Stock-solution C (Macroelements III)
2.8	$FeSO_4.7H_2O$	Stock-solution E (Fe-EDTA)
3.8	Na_2 - EDTA	
2.23	$MnSO_4.H2O$	
0.618	H_3BO_3	
0.083	KI	
0.086	$ZnSO_4.4H2O$	Stock-solution D (Microelements)
0.03	$Na_2Mo_4.2H2O$	
0.03	$CuSO_4.5H2O$	
0.003	$CoCl_2.6H2O$	
0.25	Thiamin-HCl	
0.13	Nicotinic acid	
0.13	Pyridoxin-HCl	Stock-solution F (vitamins)
0.5	Glycin	
50	Myoinositol	
50	Ascorbic acid	Stock-solution G (antioxidant)
0.018	AIA	Stock-solution H (auxin)
0.023	BAP	Stock-solution I (cytokinin)

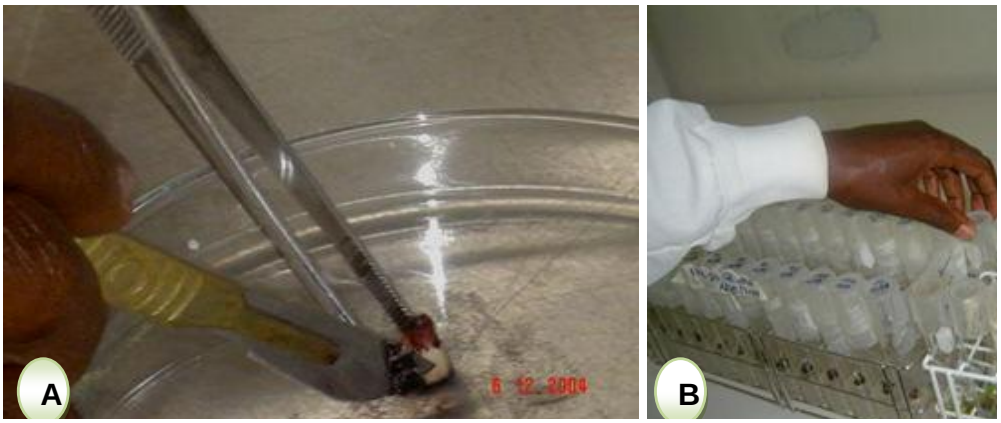

Figure 1. Excision and incubation of banana shoot-tip explants. (A) The excision was done using sterile pincers and scalpel armed with a lancet. The outer parts of the explants were delicately removed; (B) The well cut explants of banana were put on solidified MS medium contained in hermetically sealed tubes and incubated in growth chamber under controlled growth conditions (temperature, photoperiodicity and luminosity).

Table 2. Quantities (ml) taken from beforehand prepared stock-solutions to prepare the four culture media used for banana *in vitro* culture and final concentration of culture media in BAP.

Stock-solution	Volume taken from stock-solutions (ml)			
	CM1 (Control)	CM2	CM3	CM4
A	15	15	15	15
B	15	15	15	15
C	15	15	15	15
D	10	10	10	10
E	10	10	10	10
F	1	1	1	1
G	1	1	1	1
H	1	1	1	1
I	0 - - -	- 1 -	- - 10 -	- - - 100
Concentration in BAP	0 µM	1 µM	10 µM	100 µM

Table 3. Day-solutions for the five culture media used in *P. graveolens in vitro* culture and their respective concentrations in growth hormones.

Culture medium (CM)	Chemical composition	
	Common chemical composition	Specific chemical composition
1	Ms supplemented with vitamins	1 mM BAP + 1 mM NAA + 1 mM GA_3
2		10 mM BAP + 1 mM NAA + 1 mM GA_3
3		100 mM BAP + 1 mM NAA + 1 mM GA_3
4		10 mM BAP + 10 mM KIN + 1 mM NAA +1 mM GA_3
5		100 mM BAP + 100 mM KIN + 1 mM NAA +1 mM GA_3

cultures was systematically followed and the results were statistically analyzed. We controlled the changes of the culture medium, (pH variation, microbial infections and blackening) and the evolution of explants under controlled growth conditions (change of color, size and number change and quality of appeared shoot buds). Throughout this experimentation, we noticed that the pH decreased from 5.8 to 5.3 due to the culture media autoclavage whereas it decreased from 5.3 to 4.7 in one month old inoculated culture media. For the assessment of the culture media asepsis, the inspection of culture media was made after their preparation and inoculation. The autoclaved and solidified culture media were subjected to rest for the period of 3 days before it was used. On both autoclaved and used culture media, no case of infection was remarked. As far as the blackening was concerned, on the culture media which held the explants of FHIA-25 (Figure 2) and Injagi, the blackening was intensely expressed more than the blackening observed on the culture media that held the explants of Kamaramasenge and Bluggoe.

Besides the evaluation of the pH, microbial infections and blackening of the culture media, the evolution of the cultures was monitored and the main changes of explants appearance and number were recorded and then compared. The culture medium without BAP served as negative control [control (-)], the explants of all the cultivars did not give the buds, instead, they undergo necrosis even before they were subcultured. A small exception was observed on the explants of FHIA-25 which resisted until the second half of the first subculture. During the first two months of incubation of the cultures, the explants did not change their appearance perceptibly. On the medium with 1 µM BAP, the 12-weeks old explants swelled up and turned greenish and up to 2 nodule-shaped buds appeared on the contour of the explants of each cultivar. As of the third month of incubation, the buds increased in the size and in number

Figure 2. Two-month old explants of FHIA-25 inoculated on the culture medium without BAP. The culture medium turned black and the vitroplants died of tissues necrosis.

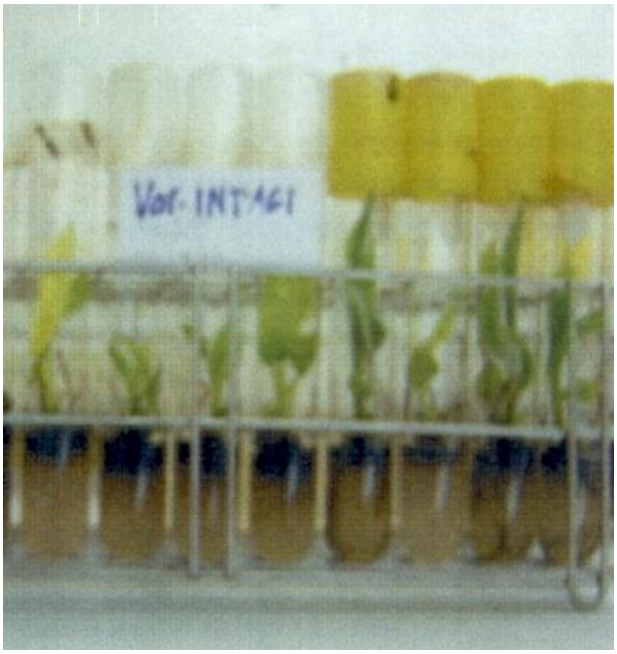

Figure 3. Three-month old explants of Injagi inoculated on the culture medium without 1µM BAP (negative control). The blackening of culture medium reduced little by little and the low ration cytokinin/auxin (1:1) favored the development of terminal (apical) shoot buds.

Figure 4. Three-month old explants of Bluggoe inoculated on the culture medium with 10µM BAP. The blackening of culture medium disappeared and the high ration cytokinin/auxin (10:1) favored the development of lateral shoot buds arising directly from green calli.

for some cultivars and developed properly, and became the healthy green vitroplants as shown in Figure 3.

On the culture medium with 10 µM BAP, the evolution of the cultures became increasingly interesting. From the 10th week of *in vitro* incubation, the explants of the cultivars Bluggoe, Injagi and FHIA-25, all green swelled up remarkably and the first green nodules appeared on their contour. At the end of the 15th week, the nodules increased their size and 1 to 3, 1 to 3, 3 to 6 and 8 to 11 shoot buds appeared on the explants of Kamaramasenge ,FHIA-25, Injagi and Bluggoe, respectively. A part of the results is presented in Figure 4.On the medium with 100 µM BAP, the results were rather exciting. After 13 weeks of incubation, all the explants increased their size and they all turned green. Specially, a great number of white and translucent nodules appeared on the contour of explants of Bluggoe. Given that they finally evolved into entire and healthy vitroplants as from the 20th week, it was undoubtedly the embryogenic calli (Figure 5). Quite the reverse, the nodules which appeared on the explants of Kamaramasenge were green and evolved directly in shoot buds as from the 15th week without forming calli. On this culture medium, 1 to 4, 4 to 9, 12 to 17 and 11 to 19 shoot buds appeared on the explants of Kamaramasenge, FHIA-25, Injagi and Bluggoe, respectively.

The results of this study were used to build the histograms below according to the average number of the vitroplants appeared on each culture media (Figure 6) and the average number of vitroplants appeared on each genotype of the cultivars (Figure 7).

According to the observations made from the twelfth week of incubation, it was noticed that the explants cultivated on the culture media with 1 µM of BAP bud quickly and provided a very low number of shoot buds as compared to those cultivated on culture media with 10 µM and 100 µM BAP. According to the same observations, the development of nodule-shaped buds into vitroplantlets was slow on the culture medium with high amount of BAP. The results of this study gave also other additional information as far as the difference of *in vitro* growth of four cultivars is concerned. We noticed that the vitroplants appeared on explants of injagi and Kamaramasenge were more or less long and were etiolated, and with small leaves contrary to the vitroplants of FHIA-25 and Bluggoe which were generally giant and short with wide leaves. The average number of vitroplants developed by every explant of each cultivar and on

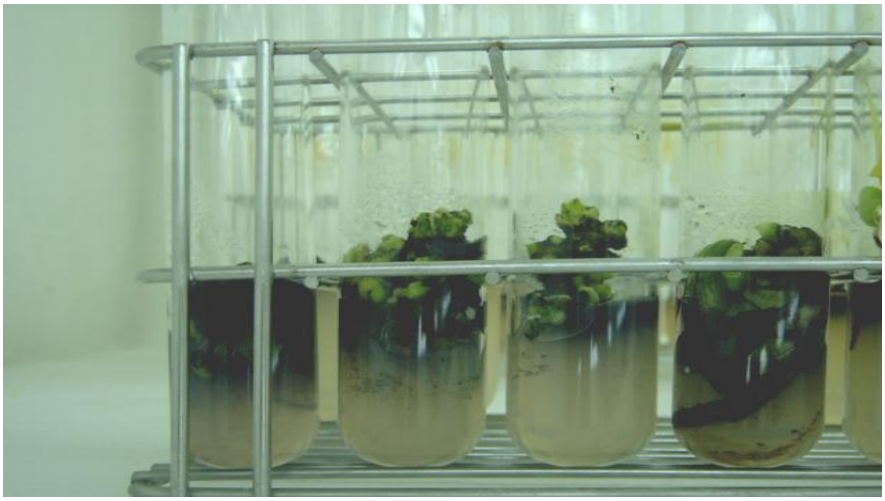

Figure 5. Three-month old explants of Bluggoe inoculated on the culture medium with 100µM BAP. The blackening of culture medium reduced and the very high ration cytokinin/auxin (100:1) favored the development of many lateral green nodule-shaped buds from embyrogenic calli.

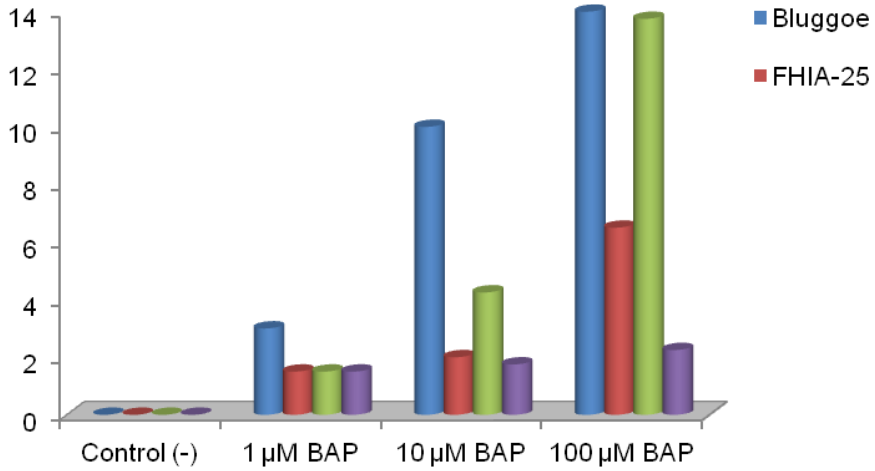

Figure 6. Comparative *in vitro* proliferation capacity of the four banana cultivars fed with four different quantities of BAP. Their capacity is directly proportional to the average number of healthy explants they allowed to produce and is represented by the length of corresponding histogram's peaks.

each culture media allowed us to draw a table of averages and variances up that helped as in ANOVA II to analyze the variability of the capacity of *in vitro* proliferation that we observed in banana cultivars.

Statistical calculations of the results of this study by ANIOVA II with the risk of 5% (α = 0.05) and 1% (α = 0.01) showed that F_{table} were very smaller than F_{obs} and led us to reject the null hypothesis (Rho**), and positively verify the opposite or alternative hypothesis. Therefore, the analysis led to conclude that the genotype of thebanana cultivars, the concentration of the culture media in BAP and their interaction have a highly significant influence on the *in vitro* proliferation rate of explants (Table 4).

Response of *P. graveolens* to the different cytokinins

The results of this study are permitted to contribute to the identification of the convenient culture medium for good *in vitro* plant regeneration of *P. graveolens*. For the disinfection of the explants, the method of alcohol

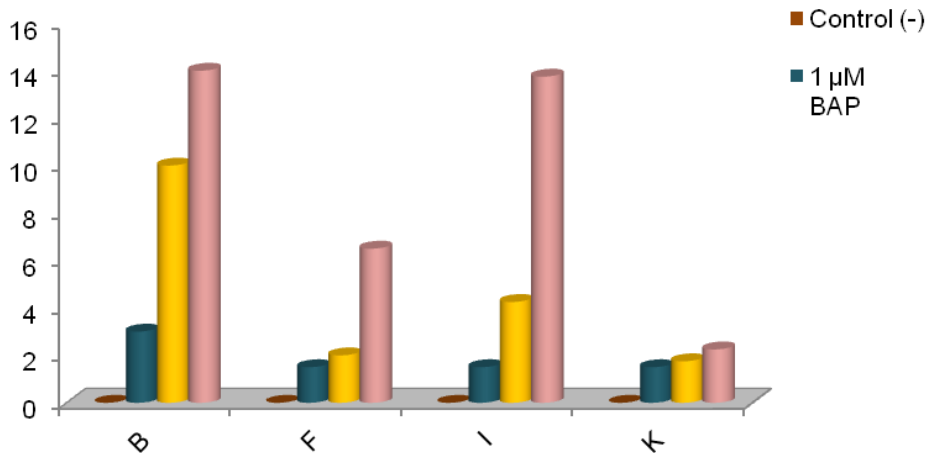

Figure 7. Comparative *in vitro* proliferation rate of the four different culture media in making the four banana cultivars proliferate. Their proliferation rates are directly proportional to the average number of healthy explants they produced and are represented by the length of corresponding histogram's peaks.

spraying followed by the soaking of explants in the calcium hypochlorite solution 5% (w/v) was the best. In this study, other alternatives used for disinfection made the explants more fragile and unable to proliferate on culture media. As far as the role of cytokinins, the culture medium MS enriched in vitamins and supplemented with 100 mM BAP + 1 mM NAA + 1 mM GA_3 was the best while addition of 10 and 100 mM kinetin hindered somehow the lateral budding and favored rather the rooting. The results of this study are permitted to compare the contribution of both the BAP and kinetin to the *in vitro* growth of *P. graveolens.* The average number of buds on each culture medium and the morphology of appeared buds are clearly presented in Figures 8 and 9 respectively.

DISCUSSION

The reduction observed in autoclaved culture medium was often due to the loss of quantity of water contained in the culture medium on the one hand and the denaturation of several components of the culture medium on the other hand by the heat. For 9 months old shoot-tip explants, culture media was used and it resulted to secretion of the chemical products in the culture medium by explants and a progressive exhaustion of some components of the culture media at the same time. Our assessment showed that the pH variation in autoclaved and used culture media was in normal range and provides our study with full trust. This change was observed in several previous studies. A reduction of the pH of 0.5 to 1.0 units for the autoclaved and hermetically-sealed culture medium and a pH of 4.5 ± 0.2 for the used culture media were previously reported (Vuylsteke, 1998). The blackening of used culture media observed in the first phase of culture of banana was due to the presence of phenolic compounds secreted by the wounded plant tissues of banana cultivars which, when oxidized, made the culture media black and the same observation was reported in previous studies by some authors (Vuylesteke, 1998). According to this results, the explants of FHIA-25 and Injagi released much exudations compared to the explants of Bluggoe and Kama-ramasenge and the degree of blackening of the culture media followed this order. From these observations, we deduced that the intensity of culture media blackening was directly proportional to the quantity of phenolic compounds exuded by explants and that the cultivars FHIA-25 and Injagi possess many phenolic compounds when compared with Bluggoe and Kamaramasenge. On blackened culture media, the bases of explants became compact and did not allow the good growth of explants. In agreement with the reports of the previous studies, the phenolic compounds secreted by explants in the culture medium and finally oxidized interfered with the elements of the medium and disturb the growth of explants (Mohan and Swennen, 2004).

The evolution of explants on the culture media gave more information about several phenomena. During the first days of incubations, the explants did not express any visible change. This month would correspond to the period of their adaptation to the new living conditions (artificial culture media) to which they had not been accustomed. The appearance of the first morphological changes after two months of incubation explains the capacity of each cultivar to adapt itself to the culture media and the speed of their *in vitro* multiplication. Therefore, this study shows that the cultivars Bluggoe (ABB), Injagi (AAA) and FHIA-25 (AAAB) exhibit a high

Table 4. Statistical calculations using various formulas of ANOVA II and conclusions on the influence level of each parameter to the *in vitro* proliferation of the four banana cultivars.

Source	SCE	dl	CM	F_{obs}	F_{table}	
					A = 5%	α = 1%
Total	1019.82	47	-	-	-	-
Residual	70.87	36	1.97	-	-	-
Factorial	1047.92	11	95.26	48.35	3.36 RHo*	4.10 RHo**
Concentration in BAP	462.37	2	231.18	117.35	2,07 RHo*	2.63 RHo**
Genotype	432.94	3	144.31	73.25	2.40 RHo*	3.00 RHo**
Interaction BAP/genotype	152.61	6	25.43	12.91	2.81 RHo*	3.60 RHo**

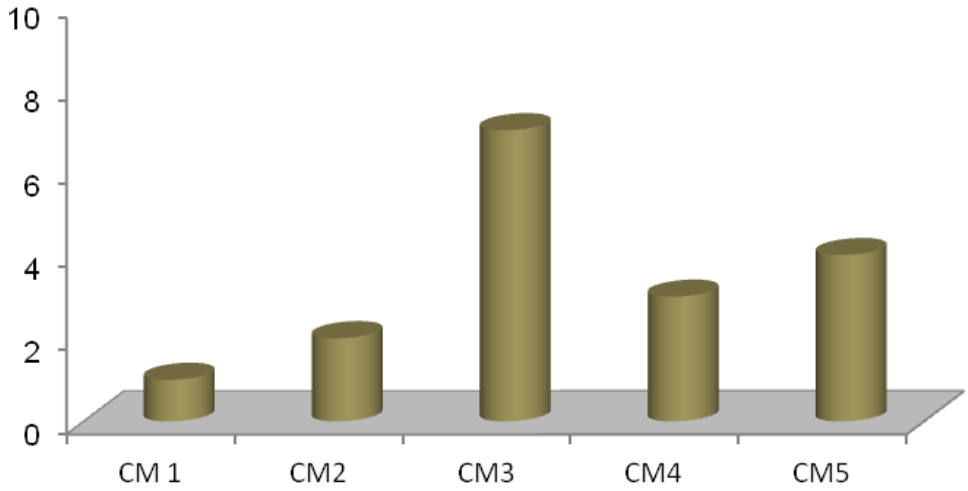

Figure 8. Comparative *in vitro* proliferation rate of *P. graveolens* ob culture media with different concentration of BAP and kinetin used as cytokinins. Their contribution is directly proportional to the average number of healthy explants they allowed to produce and is translated by the length of corresponding histogram's peaks.

Figure 9. Morphogenic response of shoot-tip explants of *P. graveolens* on MS medium supplemented with different various plant growth regulators. Proliferation of shoot buds on MS + 100 mM BAP + 1 mM NAA + 1 mM GA_3.

capacity adaptation to the artificial media whereas the cultivar Kamaramasenge is easily stressed by artificial living conditions. On this issue, this observation would bring the new idea of the possibility of specific studies on the link that exists between the genotype of a banana cultivar and its level of adaptability to artificial culture medium. From what we learned from the observations we made as from the 12th week of cultures incubation, the apical buds developed quickly on the medium with 1µM BAP. According to previous scientific reports on this point, the high ratio cytokinin/auxin supports the development of the lateral buds and inhibits the rooting whereas the law ratio cytokinin/auxin low supports the rooting and the apical budding (De Almeida and Shepherd, 1999). This was the true cause of the development of the apical buds and the roots we observed on the medium with small ratio BAP/AIA (1:1) and the opposite case on the medium with ratio cytokinin/auxin (10:1 and 100:1). In agreement with the other authors' conclusions, the elevation of the concentration in BAP had a negative effect on *the in vitro* multiplication of banana cultivars we subjected to this study (Mohan and Swennen, 2004). However, it would be careless to make such conclusion for all cultivars without doing deep studies relating to this issue in order specify the bounds of adequate concentration in BAP and to calibrate the speed of buds development.

According to histograms built on the basis of the average numbers of buds appeared on the explants of each banana cultivar, we noticed that the cultivar Bluggoe exhibits a high *in vitro* proliferation rate compared with other cultivars and even proliferate on the mediums with low concentration in BAP. This observation thus described Bluggoe as the easiest cultivar, among four studied cultivars, to be multiplied i*n vitro* in order to satisfy the need of the consumers and farmers in its plant materials. The intensification of this variety of banana would contribute effectively to the insurance of food safety in Rwanda. As far as the others three cultivars are concerned, the results showed that Kamaramasenge is stressed by the *in vitro* culture using the shoot-tip as plant material. However, other techniques including the plant regeneration from the embryogenic cell suspensions can be successfully used to multiply the Kamaramasenge cultivar (Strosse et al., 2006; Panis et al., 1993). It has also shown that there is no remarkable difference between the rate of *in vitro* proliferation of Injagi and Bluggoe on the culture media with high concentration in BAP. The cultivar Injagi was shown by this study to be a banana cultivar of triploid group completely *acuminata* (AAA) likely to be multiplied *in vitro* in Rwanda. In the same way, the cultivar FHIA-25 proliferated well on the culture media with high concentration in BAP. Although *the in vitro* multiplication of Injagi and FHIA-25 seems to be economically non beneficial, because it uses a great quantity of cytokines, it must be favored. Thanks to their good qualities.

The particular cases were listed for *P. graveolens* explants. The embrittling of explants observed during the disinfection with alcohol and other solutions was due to the duration of treatment on the one hand and the chemical nature of the solution on the other hand. Thus, the steeping of explants in an ethanol solution was described by this study as an inappropriate method for the disinfection of explants of P*. graveolens*. In the same way, the solutions of sodium hypochlorite (3.5%), mixed with Tween 80 and mercury chloride (2%), damaged and embrittled the explants, and the alternative which is using low concentrations did not allow good disinfection. This fragility would be due to the excessive effect of Tween-80 which is a liquid detergent used to lower the superficial tension of vegetable tissues in order to reinforce the penetration of disinfectants (Vuyesteke, 1998). The same effect would be allotted also to mercury (Hg) contained in the mercury chloride solution which damaged also the explants of *P. graveolens*. The cases of explants yellowing observed in some stages of this study testified the progressive decrease of some chemical compounds including magnesium of the medium. Indeed, Mg is a compound used by the plant during chlorophyll formation. In this study, we also noticed that the combination of high concentrations of BAP and kinetin favored a low lateral budding of the explants and good rooting. This effect would be due to the phenomenon explained and reported by MOHAN and his collaborators and observed at the banana (Mohan and Swennen, 2004).

This study arrived to the majority of the situations reported by previous studies but it brought new and complementary information to them. The statistical analysis of our results clearly permitted to lengthening the list of the parameters responsible for the difference in capacity of *in vitro* proliferation of four cultivars subjected to this study. Vuylsteke reported that the genotype of the cultivars and the concentration of the culture medium in BAP belong to the parameters which influence the *in vitro* regeneration rate of banana (Vuylsteke, 1998). However, this investigation revealed the significant implication of the combined effect of these two parameters, the genotype and the concentration in BAP, as the third parameter that influences the capacity of *in vitro regeneration* of banana cultivars. Moreover, the studies undertaken on several cultivars of banana showed that the great *in vitro* proliferation was linked to the character B "*balbisiana"* (Banerjee and al., 1987). With the same point of view of Zamora, our study has somehow contradicted Banerjee and his collaborators with examples as support. Our study noted the cultivar Injagi, a cultivar completely *acuminata* (AAA) has a high capacity of *in vitro* proliferation compared with Kamaramasenge with the character *balbisiana* (AB). In addition, we noticed that the cultivars FHIA-25 and Kamaramasenge proliferate differently *in vitro* whereas

they present the same degree of character "*balbisiana"* (Daniells et al., 2001). With regard to *P. graveolens*, the information provided by this study seems to be insufficient and therefore more thorough studies are necessary considering that we have few varieties of this plant in Rwanda. This present study undertaken on four cultivars of banana highly appreciated in Rwanda contributed to the policy of the use of technology in agriculture and integrated management of phyto-pathogenes via the enrichment of knowledge on the possibilities of multiplying and of regenerating the plants of some cultivars banana and geranium at the laboratory level.

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