

Mass Production of Bananas and Plantains (*Musa* spp.) Plantlets through *in vitro* Tissue Culture Partway: A Review

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ABSTRACT

Bananas and plantains are among the most important food crops in Central and West Africa. Their plantation is lead to many problems. In the recent decades, biotechnology tools using *in vitro* culture technics are used for the mass and free disease plantlets production in order to increase the bananas production and the yield. The main way of *in vitro* tissue culture at this end is the direct organogenesis i.e., the ability of plant tissues to form various organs de novo by shoots or roots induction to differentiate from a cell or cell clusters. This review aims to summarize the main results obtained in the organogenesis of bananas and plantains (*Musa* spp.) under *in vitro* conditions and to identify the challenges during the process. The research articles used in this review show that micropropagation is a reliable alternative to conventional production system of bananas and plantains planting material. However, the use of the *in vitro* micropropagation for bananas and plantains entails choosing the optimal explant type and size according to objectives. Benzylaminopurine remains the preferred cytokinin for *in vitro* banana and plantain shoot proliferation, while the use of thidiazuron appears to be more and more common. Whichever cytokinin used, the optimal cytokinin concentration for shoot proliferation is genotype dependent. This review also focuses on the causes and control measures of the two major banana and plantain micropropagation constraints: lethal tissues browning/darkening and microbial contaminations. It showed that applying the suitable and available control measure, according to the evolution of culture, is necessary. All this available information on the *in vitro* conditions makes banana and plantain cultivars *in vitro* organogenesis possible.

Keywords: explant asepsis maintenance, growth regulators, lethal browning control, microbial contamination, *Musa* spp.

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I. INTRODUCTION

Bananas and plantains (*Musa* species) are among the five major food commodities in the developing world. In banana and plantain, the difficulty to obtain large number of uniform disease free plants by the conventional propagation of techniques is one of the major limiting factors in increasing

productivity. Tissue culture technology enable the rapid production of a large scale of uniform disease free plants from a single plant. Micropropagation of banana and plantain began in the mid-1970s. Today, it is well established and commonly used by commercial firms for industrial production of healthy planting material. Direct organogenesis, multiplication, and *in vitro* regeneration of plantlets from meristems or apices without callus induction,

remains the pathway most used by commercial firms for the banana *in vitro* propagation. This pathway offers the potential to produce a large number of homogeneous and genetically identical plants [1]. Much research has been devoted to the organogenesis of bananas and plantains for more than four decades and new studies continue to be published. This review focuses on distinguishing between what already belongs to research achievements in the domain and production challenges. The aspects discussed in this review will give the state-of-the-art with respect to the type and size of the explant, its surface sterilization, the role of exogenous plant growth regulation, the browning due to oxidation of phenolic compounds and the contamination from pathogens such as endogenous bacteria.

II. INFLUENCES OF THE TYPE AND SIZE OF EXPLANT

For direct organogenesis of bananas and plantains, all parts of the plant containing a meristem are potentially suitable as initial explant. Therefore, buds from the main pseudo stem, its suckers, peepers or male inflorescence apices and immature male flowers are used as explants [2]. The influences of the type of explant, its size and the method of its sterilization are discussed in this section.

The organ used as explants can be classified into two types: stem buds and floral buds. Stem buds are the most preferred for *in vitro* tissues culture of bananas and plantains. This includes apical and axillary buds of young suckers as well as the lateral buds of the parental pseudo stem. They are easy to handle and damage to the parent plant during their removal is limited, when sampled from suckers [3]. Shoot tip from young sucker is the mostly used explant [4]. It promotes rapid multiplication and possible sanitation of plant material. It is highly protected by the superposition of leaf sheaths, which facilitates its survival during disinfection and during culture [5]. However, it allows only one explant per sucker [6], which can be a handicap if the number of suckers to be taken in the field is not sufficient to satisfy the important needs of vitroplants [7]. Little work has been published on axillary buds of suckers. However, their use allows to increase the number of initial explants per sucker. Three to four axillary buds can be removed from each sucker against a single apical bud [7]. *In vitro* proliferation of the axillary bud occurs earlier than shoot tip [8], but the survival rate after induction is low as compared to shoot tip [7]. This can be partly explained by the report of a poorer endogenous auxin of the axillary buds compared to the apical one; as there is an endogenous auxin gradient that decreases from the apex to the periphery [9]. For our understanding this theoretical difference in endogenous hormone content between axillary and apical buds could induce many differences in the response of the two types of explants during their culture. Hence, the addition of auxin to the initiation medium should be tailored to axillary buds to improve their survival rate. Studies are needed to confirm this hypothesis. The stem explants have the disadvantage of being more exposed to telluric microorganism contamination. In particular, the activities of the endogenous bacteria of these explants appear to be one major constraint of the micropropagation of banana and plantain [10].

The second type of explants are male inflorescence apex, axillary young flower buds, and terminal floral apex. In

French banana, terminal inflorescence apices and young flower buds in bract axils are capable of regenerating plantlets *in vitro*, similar to the shoot-tip culture technique [11]. It is the case in false horn-like plantains, which do not have a terminal apex and terminate with a floral structure [12]. The terminal floral apex and the axillary flower buds show morphogenic plasticity in their embryonic stage and can be induced to revert to vegetative meristems, multiplying *in vitro* and consisting a good starting material for micropropagation [2]. The apices can be used whole as a single explant [13] or split longitudinally into four to six parts [14], [15] while immature male flowers are used as a whole for explants. The use of this type of explant offers the opportunity to select *in situ*, a male bud from a bunch showing elite or highly desirable characteristics such as a large number of hands and fruits [1]. It is also an alternative in any cases where suckers were not available [14]. Moreover, since it is mostly not contaminated by telluric microorganisms, floral explants allow to reduce contamination rate during micropropagation, as compared to soil grown suckers [16]. Despite its potential, the proliferation rate of floral explants remains lower compared to stem explants [17].

According to the size of the explant, several reports revealed that the use of various sizes depends on the purpose of the culture. Ended, for the possible eradication of any bacterium and any virus, the meristematic dome surrounded by one or two primordia is used for the culture [18]. While for just propagation, the size of the explant generally varies between 2 and 5 mm, up to 10 mm or even more [19], [20]. Meristem dome culture leads to slower regrowth and high mortality [21] following rapid explant dehydration, which is not the case for large explants. On the other hand, large explants can have lateral buds that provide shoots for multiplication. But the risk of loss by infection and blackening is higher, leading to final survival rate even lower than when meristem culture is used [4]. Hence, optimal size must be taken into account for both survival and proliferation optimal rate.

III. EXPLANTS SURFACE STERILIZATION PRIOR *IN VITRO* CULTURE OF BANANA AND PLANTAIN

A critical aspect for the *in vitro* culture process is the maintenance of asepsis [21], [22]. It is partly obtained by disinfecting the surface of the explants. Disinfection requirements depend on the type of explant. Explants from inflorescence or flowers are potentially less contaminated with telluric microorganisms. Therefore, a short rinse with ethanol (70°) can be sufficient for their disinfection [17], [14]. On the other hand, stem explants more exposed to telluric microorganisms require the use of more effective disinfectants to successfully carry out their surface sterilization. Disinfectants commonly used in this context are sodium hypochlorite, calcium chloride, mercuric chloride, and ethanol.

Calcium chloride is used at a concentration of 1.5% [23]-[25], whereas mercuric chloride is often used at a concentration of 0.1% [26], [27]. In general, sodium hypochlorite (NaOCl) is used at a concentration ranging from 0.25% to 1% [5], [4], [28]. This disinfectant has proven very effective effect against all kinds of contaminants [29]. At a

concentration of 1%, sodium hypochlorite would be a better disinfectant than the mercuric chloride used at a concentration of 0.1% for 5 minutes [3]. In the absence of a laboratory NaOCl solution, commercial bleach is equally effective [30].

The chloride solutions are used in combination with a wetting agent (Tween 20, Tween 80 at a rate of one drop per 50 ml of solution) or liquid soap to improve the action of the product [31]. The duration of immersion of the explant in the solution varies from 6 to 30 min according to the authors [25], [28]. Generally, immersion in the chloride solution is preceded by rinsing (15 seconds to 2 min) in a solution of ethanol (70° to 96°). Some authors recommended double disinfection: firstly, of large blocks of tissue that contains the meristem (about 4×2 cm) followed by another of a small block of 2×1 cm [5]. This double disinfection allowed to reduce the contamination rate during the culture according to certain works [32].

IV. MICROBIAL CONTAMINATION CONTROL DURING *IN VITRO* CULTURE OF BANANA AND PLANTAIN

Microbial contamination is one of the major challenges of plant micropropagation [87]. The main microbial agents that develop in plant tissues culture are bacteria and fungi. *Pseudomonas syringae*, *Bacillus licheniformis*, *Bacillus subtilis*, *Cornebacterium* sp. and *Erwinia* spp. have been reported as the primary bacterial contaminants in plant tissue cultures [88], while *Alternaria tenuis*, *Aspergillus niger*, *Aspergillus fumigatus* and *Fusarium culmorum* are the most commonly observed fungal contaminants [88], [89]. These organisms cause significant losses at initiation and during subcultures, with each subculture contributing 5 to 15% of losses [90]. In bananas and plantains, the overall losses caused by microbial contamination are estimated at 40-60%, despite the use of reliable aseptic procedures [91]. Thus, a large number of explants are lost during culture due to microbial contamination [10].

Microbial contaminations are favored by *in vitro* culture conditions that induce a degree of stress and thus predispose the biological material to direct infection, even by microbes that normally are not pathogenic to it [92]. The culture media on which the plant tissue is grown is also a source of nutrients favorable for microbial growth, both the original constituents of the medium and the exudates of the plant cells. Thus, pathogenic, endophytic, epiphytic, or accidental contaminants can all develop, compete with plant tissues for nutrients, and interfere with their growth [93]. In addition, some of these contaminants produce phytotoxins that induce tissue necrosis, inhibit shoot proliferation and rooting. For example, *Aspergillus niger* and *A. flavus* have been reported to produce poisons such as oxalate and aflatoxin [94].

The major cause of microbial contamination that occurs during tissue culture process is imperfect sterilization of the explants, culture medium, work instruments, and operator's hands [90]. Generally, sterilization of the explant removes most epiphyte contaminants but not endogenous contaminants [10].

Endophytic bacteria are known to colonize intercellular spaces in various plants, with roots as the main entry point and niche [95]. In bananas, these endophytic bacteria also

colonize intracellular spaces, including cytoplasm and the confined peri-space between the cell wall and the plasma membrane with a large number of organisms in both niches [96], [97]. It is recognized that all plants harbor endophytic bacteria, which are essentially harmless to the host, with a smaller population than pathogens [98]. During *in vitro* tissue culture, these endogenous bacteria escape into the nutrient medium, and grow in colonies large enough to compromise the culture [99]. They are therefore potential contaminants and modifiers to the growth of plant material in culture [100]. Work has shown three groups of endophytic bacteria associated with banana cv. Great Dwarf. While endophytes in the first group are easily cultivable and develop obvious colonies on Murashige and Skoog (MS) basal medium, those in the second and third groups first appear to survive in a viable but non-culturable state and then exhibit progressive "activation" of the cultivable form as the *in vitro* culture continues [101], [87]. This could partly justify the appearance of contaminations on material that has already undergone several subcultures without contamination.

A. Control of the Activity of Endogenous Bacteria

Contaminant control is provided by adding a specific dose of an antibiotic or a specific fungicide to the culture medium [91]. These products can also be used in pretreatment solution of explants before their introduction *in vitro* [10]. Hence, gentamicin, rifampicin or chloramphenicol added to the culture medium at a dose of 150 mg/l [91], cefotaxime at 500 ppm [102] have been revealed to be effective against the endogenous bacteria of the banana. By soaking the explants for 100 min in a gentamicin solution at 160 mg/l prior to *in vitro* introduction, 100% contamination-free cultures have been obtained [10]. Sometimes the combination of several antibiotics is needed to fight infections. But these antibiotic products are often bypassed by bacteria that become resistant [99]. They can also interfere with the growth of the explant. Other researchers have used antiseptic substances to control microbial contaminations *in vitro*. Banana-free cultures has been obtained by adding nanoparticles of Zn and ZnO to the culture medium, without any negative effect on regeneration [92]. The Application of systemic fungicides prior to plant material removal may also help to suppress these contamination [103]. Finally, the sampling of the suckers followed by the initiation of the explants during the dry season rather than in the rainy season on the one hand, the reduction of the content of the culture medium in sugar on the other hand are all effective measures of reducing or elimination microbial contaminations during banana and plantain tissues culture [22].

V. INFLUENCE OF GROWTH REGULATORS ON BANANA AND PLANTAIN MICROPROPAGATION

In plant cells, tissues or organs culture, growth regulators are crucial components of culture media as they influence the cell development pathway [33]. Cytokinins and auxins have important roles. Auxins are involved in root development while cytokinins favor shoot formation and bud regrowth. The combination of these growth regulators is necessary for *in vitro* culture media, and the ratio auxin/cytokinin needs to be considered to successfully modify the growth pattern of

the plant material during the culture [34].

Firstly, cytokinins are plant growth regulators that control plant growth and development processes, such as cell division, elongation, and differentiation, seed dormancy and germination, leaf expansion and senescence, lateral buds growth and de novo bud formation, chloroplast and aerial organ development, morphogenesis [35], [36]. At high concentrations (1 to 10 mg / l), they can induce the formation of adventitious buds, but root formation is inhibited [37].

The natural cytokinins are derivatives of adenine, mainly zeatin but also isopentyladenine, kinetin, benzylaminopurine [38]; some of its derivatives that are also topolins [39]. Many phenylurea derivatives exhibit cytokinin activity in biological experiments but are not known as natural cytokinins. One of them is thidiazuron, a highly stable compound [40] and widely used now as a plant growth regulator.

For banana and plantain *in vitro* tissues culture, the mostly used constituent of culture medium for bud formation is clearly cytokinin [2]. Apart from the genotype, the rate of proliferation is influenced by the type of cytokinin and its concentration [41]. Benzylaminopurine is the most commonly preferred cytokinin [42], [43]. It is reported to be more effective on *Musa* spp. *in vitro* organogenesis than kinetin, isopentyladenine and zeatin [44]. Each cultivar appears to be reactive to an optimal concentration of benzylaminopurine, beyond which the rate of proliferation cannot be increased and may even decrease. Concerning the stem meristematic explants, the concentration of 22.2 μM was indicated by several studies as optimal for the proliferation of most banana and plantain cultivars [42], [41], [45], [46]. Though, other values have been reported in other studies (Table I). Regeneration of male flowers and their *in vitro* multiplication are also influenced by cytokinin concentration [15]. Several concentrations of benzylaminopurine were also reported as indicated in Table I.

Despite its advantages, the use of benzylaminopurine has some drawbacks. At high doses, BAP inhibits shoot multiplication and elongation [47], [48], increases exudation of phenolic compounds [49] and favors the appearance of abnormal forms [50]. The toxicity of benzylaminopurine appears to be higher than other cytokinins. The use of benzylaminopurine in the culture medium needs to be carefully regulated to optimize proliferation and produce normal plantlets [33].

Thidiazuron, urea-based cytokinin, can allow better multiplication rate than benzylaminopurine [51]. The doses used for optimal proliferation are generally low as compared to those required for BAP [52], [53]. An exception was reported (benzylaminopurine favoring better organogenesis than thidiazuron) in proliferation for the cultivar 'Kluai Hin' of *Musa balbisiana* [54]. The doses of thidiazuron used vary from 0.05 to 2.5 μM and sometimes slightly more according to the cultivars [52], [40], [55]. The use of thidiazuron in banana micropropagation could be more economical than adenine-based cytokinins [56]. But high concentrations of thidiazuron can induce appearance of abnormal forms [57]. Thidiazuron at 0.2 mg/l induces more abnormal forms than benzylaminopurine at 5 mg/l [58].

The optimal concentration of a given cytokinin for proliferation depend on the genotype. Thus, for each new genotype to be introduced *in vitro*, tests are needed to

determine the optimal concentration for proliferation. Auxins are phytohormones that control various plant responses such as cell division and elongation, differentiation, root formation, and apical dominance [36]. Natural endogenous auxin is indole-3-acetic acid (AIA). The most widely used synthetic auxins in plants tissue culture are indole-3-butyric acid (AIB), naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D) [59].

For direct organogenesis of banana and plantain, auxins are mainly involved in root formation. The most commonly used are AIB, AIA and NAA at doses generally ranging from 0.5 mg/l to 2 mg/l [60]-[63]. But it has been reported that at high doses auxin has a negative influence on root length.

Auxins are sometimes used in combination with a cytokinin to ensure bud proliferation. The interaction between different cytokinins and NAA or AIA has been used to improve the proliferation rate [64], [19], [65]. However, for 'Basrai' (*Musa* spp., AAA group), AIA (1 mg/l and 2 mg/l) combined with multiple doses of BAP (0 mg/l to 8 mg/l) did not affect shoot proliferation [66]. This could be related to the dose of endogenous auxin which itself would depend on the genotype.

TABLE I: VARIATION OF BENZYLAMINOPURINE CONCENTRATION DURING PROLIFERATION OF VARIOUS CULTIVARS

Explants	BAP		References
	Concentrations (μM)	Cultivars	
Stem bud	5.00	FHIA-17	[29]
	8.89	BARI-1	[104]
	13.32	FIHA- 21, PITA-3	[105]
	17.78	Amritasagar	[27]
	20.00	Oniaba, Apantu	[64], [82]
	22.20	Red banana	[1], [41]
Floral bud	31.08	Berangan	[16]
	35.52	Berangan, Rastali	[14]
	44.40	Ambun	[106]

VI. BROWNING / BLACKENING OF SHOOTS NECROSIS MANAGEMENT

Potential problems with banana and plantain in tissue culture include browning/darkening of the medium and excised faces of explants during the initiation and subcultures phases [2], browning of young leaf, shoot necrosis, plantlets death during the proliferation, and rooting phases [67]. These phenomena are mainly due to the oxidation of phenolic compounds [68] but probably also to the depletion of the medium in mineral nutrients or in growth regulators or both [69].

A. Phenolic Compounds Oxidation during Banana and Plantain Tissue Culture

Banana tissues are known to contain large amount of latex and phenolic compounds [70]. They are secondary metabolites present in large quantities in plants. They regulate the catabolism of indole acetic acid (AIA) [71] and thus control plant growth, cell differentiation and organogenesis. The production of phenols is stimulated when tissues are injured, leading to the activation of certain oxidative enzymes such as polyphenol oxidase (PPO). Apart from PPO, other

oxidative enzymes such as peroxidase (POD) and phenylalanine ammonia lyase (PAL), also contribute to wound induced browning. These enzymes were considered as marker of oxidative metabolism. In most plants PODs were heme-containing enzymes that catalyze the conversion of H₂O₂ to water using phenolic compounds as hydrogen donors. PAL as a branch point enzyme in phenylpropanoid pathway has a profound impact on progress of browning/necrosis of plant tissues. In addition, POD and PPO may act synergistically in enzymatic browning, because PPO may promote POD activity by generating H₂O₂ from the oxidation of phenolic compounds [72]. Oxidation of phenolic compound is a natural defense process that induce the formation of quinones and highly reactive forms of oxygen [70]. These oxidation products are toxic to infectious pathogens and pests. They may also undergo non-enzymatic auto-polymerization or covalent hetero-condensation with proteins and carbohydrates to produce colored compounds [73]. The colored compounds produced generally constitute a physical barrier against biotic and abiotic stresses. During *in vitro* tissue culture, the phenols are released into the culture medium by the wounded ends of the explant and accumulate therein. The products of their oxidation gradually penetrate the tissues and repress the enzymatic activities. As a result, they have very toxic effect on the plant tissues [74]. In addition, the colored compounds they produce surround the explant and form a barrier that prevents the absorption of nutrients [75]. Finally, the oxidation of phenolic compounds and other exudates during plant tissues culture negatively influences explant survival, plantlets proliferation and growth [76], [74].

B. Control of the Oxidation of Phenolic Compounds

There are several ways to prevent or reduce the adverse effects of the oxidation of phenolic compounds during bananas and plantains tissue culture. These include the addition to the culture medium of an antioxidant [77], [75], [78] or adsorbents such as activated charcoal [79], [80], [63]; pretreatment of the explant by immersion in an antioxidant solution prior to introduction *in vitro* [81], [68]; increase in the concentration of the medium in calcium [67], [69]; increased frequency of subcultures and periodic culture in the dark [4], [70]; drying of the suckers under shade for several days before explants isolation [82], [83].

C. Antioxidants

Antioxidants inhibit the oxidation of labile substrates and protect the tissues. The main antioxidants used in plant tissue culture to control the oxidation of phenolic compounds are ascorbic acid, citric acid, and cysteine. They are used as pretreatment solution to prepare the explant before initiation or as component of the culture medium.

Ascorbic acid has been used by several authors to control tissue browning *in vitro*. The addition of ascorbic acid at doses ranging from 10 mg/l to 150 mg/l in the medium has been indicated to reduce the phenomenon of tissue darkening [4]. This large range of required concentrations may be due to the effect of the genotype [75]. As reported, for the cultivar Mzuzu of banana, high concentrations of ascorbic acid (100 and 200 mg/l) added to the culture medium are effective in reducing tissue browning during 4 weeks of culture [68]. On the other hand, at low concentration (50 mg/l), the

effectiveness of ascorbic acid does not exceed the first week of culture. Furthermore, the preparation of the explant by immersion for one hour in 1.2 g/l solution of ascorbic acid was just as effective as the addition of 100 mg/l of this product to the culture medium [68]. In the proliferating or rooting phase, the addition of ascorbic acid to the culture medium, prevents lethal browning of plantlets as well as inhibits the oxidation process and restores plantlets already affected [77]. It is believed that the ascorbic acid absorbed by the plantlet migrates to the leaves in order to prevent the oxidation of phenolic compounds, making its presence in the culture medium to be beneficial to improvement of plantlets proliferation [77], [74]. It is recommended to add the ascorbic acid solution at the surface of the culture medium after autoclaving rather than adding it before, because it's affected by the heat. The addition of ascorbic acid to the culture medium prior to autoclaving may partially explain the ineffectiveness of the product in reducing browning observed in some cases [75], [77].

Citric acid is also effectively used to control tissue browning, darkening and death. Like ascorbic acid, citric acid can be added at a dose varying from 10 to 150 mg/l to the culture medium [4]. A 0.125% solution of a combination of citric acid and potassium citrate (4: 1 m/m ratio) has been used successfully to treat explants such as banana flower apex [81] and shoot apices of plantain [84] prior to their introduction *in vitro*. The combination of citrate and citric acid functions as a chelating agent for the ions responsible for the activation of polyphenol oxidase.

The cysteine has been proposed at a concentration of 50 mg/l to treat the explant for a few minutes before its introduction *in vitro* [4].

D. Adsorbents

Activated charcoal is the main adsorbent used for *in vitro* culture of banana and plantain. Activated charcoal is composed of carbon arranged in a quasi-graphitic form in small particles size. It has a fine network of pores with an extraordinarily large surface area on which many substances can be adsorbed. Activated charcoal is often used in plant tissue culture to enhance growth and cell development. Its main advantages are the adsorption of inhibitory substances in the culture medium, a drastic decrease in the phenolic oxidation or brown exudate accumulation, the alteration of medium pH to an optimum level for morphogenesis, the establishment of a darkened environment in medium and hence simulate soil conditions [79]. However, activated charcoal could also adsorb vitamins, cytokinins and auxins, thereby altering the concentrations of the components of the medium and subsequently influencing plant regeneration. Hence, the use of activated charcoal does not allow researchers to control the quantities of exogenous hormones that are free and really available for plant tissues [78]. During micropropagation of banana and plantain, activated charcoal is generally used to promote rooting at doses ranging from 1.0 to 2.5 g/l [4], [17], [85], [86]. Activated charcoal alone at 1.5 g/l or in combination with 150 mg/l of ascorbic acid or citric acid permitted to inhibit the oxidation of phenolic compounds and enhances plantlet growth in Grande Naine cultivar [63]. On the other hand, some authors indicated that adsorbents in general (exchange resin, anion exchange resin,

polyvinylpyrrolidone and activated charcoal) are not effective in reducing the lethal browning of *Formosana* plantlets while ascorbic acid has been effective to solve the problem [77]. These authors stated that the oxidation of phenolic compounds would occur within the leaves of plantlets, causing their browning and death. Thus, the adsorbents can help to control browning especially at the initiation where the phenolic compounds are released into the culture medium by the excised faces of the explant.

E. Other Methods of Controlling Browning

Rapid transfer of the explants to a fresh medium twice or thrice during establishment is the simplest and most effective method of protecting explants from the harmful effects of browning. During this period, the cut end of the explant becomes sealed up and the leaching of phenolic stop [70]. This also allow to avoid the invasion of tissues by the toxic products resulting from the oxidation of phenols. These subcultures can be performed every one to two weeks [4]. Keeping the fresh crops in complete darkness for a week can also help reducing the problem of browning by preventing or reducing the activity of enzymes involved in both phenol biosynthesis and oxidation [4], [70]. Drying the suckers under shade for a few days reduces the content of the material in phenolic and their exudation during establishment [82], [83].

VII. CONCLUSION

The use of *in vitro* micropropagation for bananas and plantains entails choosing the optimal explant type and size according to objectives. The *in vitro* survival of the explant strongly depends on the measures taken to control the browning / darkening of tissues and microbial contamination. In particular, the control of the activities of endophytic bacteria remains a permanent challenge for the *in vitro* culture of banana and plantain. Benzylaminopurine remains the preferred cytokinin for *in vitro* banana and plantain shoot proliferation, while the use of thidiazuron appears to be more and more common. Whichever cytokinin used, the optimal cytokinin concentration for shoot proliferation is genotype dependent. Auxins are mainly involved in rhizogenesis but they are also used in combination with cytokinins to improve the proliferation rate. All this available information on the *in vitro* conditions makes banana and plantain cultivars *in vitro* organogenesis possible.

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