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RESEARCH ARTICLE

RELEVANT CONDITIONS FOR MICROPROPAGATION BY DIRECT ORGANOGENESIS IN TRIPLOID VARIETIES (AAB) OF PLANTAIN EXPLANTS (*Musa* spp.)

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Abstract

Banana is the most cultivated crop in Colombia. It is the most important crop with respect to food security in addition to forming part of the group of Colombian food staples. In a worldwide context, Colombia ranks fifth in banana production even though in 2018 and 2019, Colombia registered drops in planted areas and production, respectively, due to adverse weather conditions. Plantain crops are currently being affected by the vascular disease called Moko, caused by the *Ralstonia solanacearum* bacterium, which leads to a significant reduction in the cultivated area and for which there is no effective type of control. The main source of infection in disease-free areas is the propagule so that *in vitro* propagation turns out to be a viable and feasible alternative for producing certified quality plantain seeds. This manuscript presents the main problems encountered in the micropropagation of AAB triploid bananas by *in vitro* culture, factors that influence direct organogenesis, and alternative solutions to different conditions that can affect the process. This review will serve as a starting point for establishing reliable propagation protocols for different varieties of plantain in Colombia.

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Introduction:-

Bananas (*Musa* spp.) are perennial crops that grow rapidly in tropical locations and can be harvested throughout the year. A banana is characterized as a giant perennial herbaceous plant containing a short rhizome, an apparent stem to which the pods are attached, the formation of a crown of leaves. In the year 2000 alone, it is estimated that an area of approximately nine million hectares was cultivated for banana growing worldwide. In 2017, approximately 20% (22.7 million tons) of worldwide banana production was marketed, becoming the most exported fruit (Voora et al., 2020; FAO, 2020). Banana cultivation is one of the most important in Colombia and because of this importance, the country has established itself as the fifth-largest producer worldwide after the Republic of Congo, Ghana, Cameroon, and Uganda, and as the fourth largest exporter after Laos, Guatemala, and Ecuador (Minagriculture, 2021). The main varieties of cooking plantain that are cultivated in Colombia are Dominico, Dominico Hartón, and Hartón. The so-called "Comino" or Maqueño plantain, used in the "snack" and starch agribusiness and desired for its sweetness, is beginning to gain importance within this group.

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Any current figure on banana marketing is only an approximation since most of the world's banana production (almost 85%) comes from relatively small plots and home gardens that escape consumption and marketing statistics. Most of these bananas are traded locally or consumed directly by the producer, which makes the fruit an item of special relevance due to its evident importance for food security (Arias et al., 2004).

Regardless of the efforts that have been made to produce cultivation viable and inexpensive banana crops, the vascular disease called Moko, caused by the bacterium *Ralstonia solanacearum* (French & Sequeira, 2001) and *Fusarium* R4T caused by the fungus *Fusarium oxysporum* f. sp. cubense, generates millions of losses in banana production since no effective eradication methods exist; in addition, the appearance of Moko has spread rapidly throughout the Colombian territory, resulting in a significant decrease in banana yields in cultivated areas.

The Colombian Agricultural Institute (ICA) has disclosed the importance of working on measures for prevention, dissemination, and control of these phytosanitary problems through the implementation of the provisions suggested by resolutions 17334 of 2019 and 92770 of 2021 (ICA, 2019; ICA, 2021).

Banana seed is the primary infection vector in disease-free areas in which one of the main prevention measures is the production of healthy seeds. In this sense, *in vitro* propagation has proven to be a viable alternative for propagation in traditional nurseries by ensuring healthy, pathogen-free germ material (Domingues 1995; Olmos et al., 2010).

Many factors affect the success of *in vitro* micropropagation of plantain, for example, the type and composition of the culture medium, micropropagation system, nature and size of the explant, explant disinfection method, and environmental conditions each of which make it necessary to adjust specific protocols according to the variety and technological resources of each laboratory (Waman & Bohra, 2019).

The objective of this work was to construct an updated documentary tool that supports decision-making in the context of the producer who suffers from the risk of possible diseases on their crops. Thus, in the document, viable alternatives for the use of *in vitro* techniques based on the objectives of the micro-propagation and the scientific evidence are discussed in addition to some relevant aspects to consider when thinking about using an *in vitro* culture. This document should be useful as a starting point for establishing reliable propagation protocols for different varieties of plantain in Colombia.

Materials and Methods:-

An information search was carried out in databases, including Scopus, Science Direct, Scielo, and Google Scholar websites. The search was carried out using keywords in English and Spanish, conjugated with Boolean operators to specify the information. The most used keywords were "micropropagation", "plantain", "banana", "*in vitro*", "AAB", "cultivation", "standardization", and "*Ralstonia solanacearum*" with the Boolean operators AND, NOT, and OR. The documents were reviewed and classified by the authors according to their relevance in meeting the objectives of this review until obtaining a base of 63 nationally and internationally published documents that were then grouped into scientific articles, books, and official reports since 1943 to date. Additionally, information was obtained from the Center for Research in Agriculture and Biotechnology (CIAB) of the National Open and Distance University (UNAD) in which experiments have been conducted out for the *in vitro* propagation of *Musa paradisiaca* since 2013.

Results and Discussion:-

Plant micropropagation techniques are based on potential cell differentiation, which assumes that any plant cell contains a complete copy of the genetic material of the plant to which it belongs regardless of its function or position in it and therefore, has the potential to regenerate a completely new plant (Calva & Pérez, 2005). Although all cells are theoretically totipotent, meristematic cells are the most capable of expressing this quality (Shamoun, 2004). The morphogenetic response of somatic cells can be manifested via one of two alternative routes: (1) organogenesis or (2) somatic embryogenesis. Organogenesis is a biphasic process during which the induction strategy for the formation of new cells results in the formation of organs from callus culture (Pérez-Bernal et al., 2008; Viñas & Jiménez, 2011). On the other hand, somatic embryogenesis (SE) is a process that involves the formation of embryos from plant somatic cells. It is noteworthy that as a result of SE these embryos have a bipolar structure with stem

apices and roots and similarity to the structure found in zygotic embryos, which makes SE an ideal technique for generating viable clones with successful adaptability independent of the medium.

Depending on the characteristics of the plant to be propagated and the objective of the said propagation, micropropagation can be carried out through three regeneration pathways: (1) sprouting of pre-existing adventitious buds, (2) production of "*de novo*" buds (differentiation within the explant that leads to the formation of primary meristems, roots, and bud primordia), and (3) somatic embryogenesis (Olmos et al., 2010). Concerning the culture medium, it is common to divide tissue culture techniques into two large groups: (1) cultures in semi-solid media and (2) cultures in liquid media, which in turn can be stationary or subject to continuous shaking or placed in temporary SIT immersion systems. It is also common to divide tissue culture according to the levels of complexity into organ, cell, and protoplast cultures (Roca & Mroginski, 1991).

For three decades, efforts have been devoted to perfecting *in vitro* propagation protocols for plantains (Vuylsteke & De Langhe, 1985). Today, each laboratory has established its protocol, depending on the varieties, including the propagation of Heliconias and other Zingiberaceae, particular clones, and environmental factors. *In vitro*, clonal propagation studies from meristem culture began in the early 1970s, and application of this method to bananas was first described by Ma and Shii in 1972 (Ma & Shii, 1972; Roca & Mroginski, 1991). Currently, bananas are propagated *in vitro* using three main techniques depending on the purpose of obtaining the seedlings: (1) culture of apical and axillary meristems, (2) culture of floral meristems, and (3) culture of protoplasts. Meristem cultures are used for mass production of plant material for commercial crops, while seedlings obtained from protoplast cultures are used for genetic improvement purposes by providing materials with greater somaclonal variability (Uzcátegui et al., 2010; Nwauzoma & Jaja, 2013).

Direct Organogenesis Regeneration from Apical Corm Meristems. Morphologically, the corm is defined as a stem that develops leaves in the upper part and adventitious roots in the lower part (Soto, 1985). Therefore, in Musaceae plants, the stem corresponds to an erect underground corm with monopod branching (Belalcázar et al., 1991) and is evident after cutting the apical meristem longitudinally at which point it can be observed that the meristem is located in a depression enclosed between the foliar surrounded by differentiated leaves (Soto, 1985). At the base of each node, the non-axillary buds are inserted in an opposite way (Soto, 1985; Belalcázar et al., 1991; Vargas-Calvo et al., 2015). The corm has many active and latent buds that are controlled by regulatory mechanisms; therefore, when the corm is divided into several parts, each of them can give rise to a plant if at least one vegetative bud is present and receives special care (Haddad, 1994).

Cultivation by apical meristems is widely used for plantain propagation and although the proliferation rates are not uniform, it allows for a good yield of many healthy propagules that are genetically stable and free of contaminating microorganisms (Gamborg et al., 1968). Through direct organogenesis from the apical meristems, the sprouting of axillary buds is obtained (Figure 1) that allows for propagation of new plants. Through indirect organogenesis, the formation of a mass of undifferentiated cells called "callus", is stimulated, which will later give rise to new shoots.

Figure 1:- Axillary meristems obtained from *in vitro* seeding of an apical meristem.

Planting Material Characteristics. Generally, it can be said that the younger and more undifferentiated the explant to be cultivated, the better its response *in vitro* will be, which is the reason for using apical and axillary meristems (Olmos et al., 2010).

Plantain explants are commonly obtained from “needle or sword” type suckers as shown in Figure 2 (Ortega et al., 2010; Sandoval-Cancino et al., 2013), dried apricots (Aranzazu et al., 2005), adult corms between 400 and 600 g (Uzcátegui et al., 2010), and meristematic domes obtained from *in vitro* subcultures (Héctor et al., 2007; Pérez et al., 2013).

Figure 2:- From left to right, spade or needle sucker and explant of the apical bud of a corm.

Depending on the size of the explant that is added to the culture medium, significant differences have been found in terms of survival percentage, oxidation, and contamination. The most appropriate explant sizes for budding are between 1 and 5 mm^3. These explants can achieve a size of up to 1 cm^3 and conserve a portion of the corm tissue. Larger sizes result in greater contamination of the cultures as it is more difficult to achieve adequate disinfection before planting, and smaller sizes result in higher rates of explant deaths due to oxidation (Canchignia et al., 2008; Hoyos et al., 2008; Villegas et al., 2008; Uzcátegui et al., 2010; Pérez et al., 2013). In the case of explants from *in vitro* plants (meristematic domes), the size ranges between 0.1 and 0.5 mm.

Disinfection of the Explant prior to Sowing. When the corms are obtained from the field, it is necessary to clean and disinfect them to remove soil residues and other contaminants. After removing all roots and submerging the corms in a soapy solution, the corms are then reduced to a size of 10 cm and submerged in a sodium hypochlorite solution (4%–20%) for the time period, which usually varies between 5 and 20 min according to the selected protocol (Canchignia et al., 2008; Hoyos et al., 2008; Sepulveda et al., 2008). The concentration of hypochlorite and immersion time varies according to the characteristics of the explants. These parameters must be adjusted in such a way that the percentages of oxidation of the tissues are reduced once seeded *in vitro* according to the criteria of each researcher. The higher the concentration of hypochlorite and the longer the immersion times are, the higher the percentages of explant loss due to oxidation will be. To counteract the hypochlorite effect, submerging explants in antioxidant solutions based on cysteine (60 mg L-1), aluminum sulfate, and ascorbic acid (50, 75, and 100 mg L^{-1}) has been successfully tested (Ortega et al., 2010; Uzcátegui et al., 2010; Rios et al., 2013) during which the explant size reduction process is performed. Disinfection and three washes with distilled water should be done before planting in the culture medium. It has been shown that when the explants are dried in the shade for either 7 or 14 days followed by immersion in a solution of 50 mg L^{-1} of cysteine, oxidation-reduction occurs; therefore, a 100% survival rate of the explant material during the first four weeks of cultivation was found to occur (Agbadje et al., 2018). On some occasions, antioxidants are also sometimes included as additional components of the culture medium (Hoyos et al., 2008; Villegas et al., 2008; Uzcátegui et al., 2010).

Based on the tests carried out on *in vitro* micropropagation of plantain at the CIAB of the National Open and Distance University (UNAD), a protocol for disinfection of apical meristems obtained from the corms of the plantain variety Dominico Hartón was adopted according to the study by Waman et al. (2015). The modification of the protocol and its variants presents 16 steps in which those between 13 and 16 must be carried out in a laminar flow cabinet to avoid re-contamination of plant material and the MS culture medium (BAP 4 mg L-1 and IAA 1 mg L-1) as shown in Table 1.

Table 1:- Explant disinfection protocol.

Step	Description	Concentration	Time
1	Initial rinse with soapy water (with the help of a brush, the surface of the plant material is washed)	-	-
2	Wash with soapy water (with the help of a brush, the surface of the plant material is washed)	-	15 min
3	Wash with tap water	-	-
4	Cut	-	-
5	Wash with Tween 80	10%	15 min
6	Wash with sterile distilled water	-	-
7	Cut	-	-
8	Immerse in bactericide (streptomycin + penicillin)	200mg L^{-1}	1 hour
9	Cut	-	-
10	Dip in broad spectrum fungicide	1,5mL L^{-1}	30 min
11	Wash with sterile distilled water	-	-
12	Immerse in Quaternary ammonium	6%	10 min
13	Immerse in sodium hypochlorite	3%	15 min
14	3 washes with sterile distilled water	-	-
15	Cut 1,5cm * 1cm * 1cm	-	-
16	Seed	-	-

The corms used for *in vitro* cultivation were obtained from a nursery with health certification from the Colombian Agricultural Institute (ICA) and reseeded in the greenhouse of the UNAD operational headquarters located in the department of Risaralda (4°49´49´´N 75 °40´13´´ W; Dosquebradas 1469 m asl).

Some authors report the use of mercury bichloride ($HgCl_2$) at a concentration of 0.1% P/P (Jafari et al., 2010; Shahnawaz et al., 2014; Waman & Bohra, 2019) or 0.2% P/P (Roy et al., 2010) within the explant disinfection protocols, such as those describing meristems obtained from the corm. The use of $HgCl_2$ offers broad viability and feasibility to the establishment of the culture since it optimizes disinfection times (contact time less than 14 min) with a survival of ≥ 80% (Canchignia et al., 2008; Shahnawaz et al., 2014; Ferdous et al., 2015).

Nature and Composition of the Culture Medium. The composition of the culture medium and its contact with the tissues and oxygenation influence the multiplication capability of the explants (Lopes de Siqueira et al., 2013). In general terms, all culture media have five main components: (1) carbon sources, (2) minerals, (3) vitamins, (4) gelling agents, and (5) growth regulators (Roca & Mroginski, 1991; Galan et al., 2018). The most frequently used culture medium for *in vitro* plantain propagation is the one developed by Murashige and Skoog (MS) because its formulation meets the nutritional requirements of the species; however, other commonly used media, such as B5 from Gamborg (Gamborg et al., 1968) that is especially used for cell and protoplast culture and contains a lower concentration of nitrates compared to MS, Chu's N6 medium (Chu et al., 1975), and WH medium (White, 1943), which is the most dilute medium of the ones described above and is therefore almost always used as a complement to MS.

In terms of consistency, culture media can be liquid, semi-solid, or solid, and the behavior of the explant in the culture medium depends on the type of tissue and the stage of development. In bananas, the initiation phase can be carried out in a liquid medium, and subsequently, the subcultures are prepared in a semi-solid medium. Liquid media are easier and faster to prepare, cheaper, and provide greater homogeneity of easily diffusing components. In this sense, a higher multiplication rate of explants in liquid medium has been reported by a stationary system with volumes of 25 mL per flask compared to other systems. The medium is periodically renewed to favor oxygenation

(Lopes de Siqueira et al., 2013). On the other hand, Lemos et al. (2001) found that liquid media, both stationary and during agitation, promotes greater explant development by allowing a greater contact of the tissues with the medium so that the nutrients can penetrate all parts of the explants (Lemos et al., 2001).

One of the limitations of liquid media is the difficulty in acclimatizing plants to external conditions, which is why this type of medium is generally used only in the initial phase of culturing. However, other researchers have found that using a SIT temporary immersion method, it is possible to propagate large numbers of plantains *in vitro* in liquid media without observing any negative effects on the resulting plants and also to cause a reduction in the high rates of tissue oxidation that the process entails. The use of a static immersion system allows for the achievement of the greater proliferation of healthy explants and automation of some of the steps in the micropropagation process. (Villegas et al., 2008; Pérez et al., 2013).

Eyebrows et al. (2011) and Basail et al. (2013) found that the highest multiplication rate and the lowest incidence of oxidative stress in Vianda plantain occurred with immersion times of 10 min every 6 h (Cejas et al. 2011; Pérez et al. 2013), while Colmenares & Giménez (2003) found the best results with 2 min immersions every 4 h with an increase in the multiplication rate between 2.6 and 3.5 times higher compared to the multiplication rate in a solid medium in the Hartón variety. These authors also found that Musaceae containing the *Musa acuminata* genome responded better to *in vitro* cultures in SIT than did the wild species, *Musa balbisiana* (Colmenares & Giménez, 2003).

Growth Regulators. Induction, growth control, and morphogenesis are influenced by the hormonal relationships used in the culture medium, associations that in turn seem to be influenced by genetic factors (Canchignia et al., 2008; Villegas et al., 2008; Rios et al., 2013), which is why the concentrations and types of hormones used in each of the development stages of the *in vitro* explant varies greatly from one variety to another. In addition, whether obtaining shoots by directly or by prior callus formation is desired is also a factor in choosing a culture medium. Table 2 summarizes some of the culture media and hormone concentrations that have been successfully tested in the micropropagation of AAB bananas.

Table 2:- Different types of media and doses of hormones according to variety and stage of development of the explant for micropropagation from apical and axillary buds in AAB plantain by direct organogenesis.

Variety	**Media type**	**Development phase**			**Cite**
		Establishment	**Multiplication**	**Rooting**	
Maqueño	Solid	5mg L^{-1} BAP + 1,2mg L^{-1} 3-IAA	MS + 20 g saccharose + 1g activated carbon	Soil	(Canchignia et al., 2008)
Dominico Hartón	Solid	MS + BAP 1mg L^{-1}+ 3-IAA 0,2mg L^{-1}	MS + BAP 5mg L^{-1}+ 3-IAA 0,3mg L^{-1}	MS + BAP 0,5mg L^{-1} + 3-IAA 5mg L^{-1}	(Hoyos et al., 2008)
Hartón	Liquid or temporary immersion system (TIS)	MS + coconut water 15% + pantothenic acid 1mg L^{-1} + Biotin 0,01mg L^{-1} + Thiamine 1mg L^{-1}	MS + BAP 5mg L^{-1} + Cysteine 60mg L^{-1} + ascorbic acid 100mg L^{-1}		(Camolesi et al., 2010)
Maçã	Solid	MS/2 for macronutrients + BAP 1,0mg L^{-1}	MS/2 for macronutrients + BAP 2,5mg L^{-1}	MS/2 for macronutrients	(Camolesi et al., 2010)
Cambur Manzano	Solid	MS + 50mg L^{-1} ascorbic acid + BAP 5mg L^{-1}	MS + 75mg L^{-1} ascorbic acid + BAP 2,5mg L^{-1}		(Rios et al., 2013)
Hartón	Solid	MS + Morel's vitamins (100mL L^{-1}) + 5mg L^{-1} BAP and 1,30mg L^{-1} IBA	MS + Morel's vitamins (100mL L^{-1}) + 5mg L^{-1} BAP and 1,30mg L^{-1} IBA	MS + Morel's vitamins (100mL L^{-1}) + 5mg L^{-1} BAP and 1,30mg L^{-1} IBA	(Uzcátegui et al., 2010)
Musa	Solid	MS/2 + Activated	MS/2 + BAP 2mg		(Sepúlveda et al.,

balbisiana		carbon	L^{-1} + 3-IAA 0,5mg L^{-1}		2008)
INIVITPV-2011	Liquid TIS		MS + BAP 2,0mg L^{-1}+3-IAA 3,5mg L^{-1} + ascorbic acid 10mg L^{-1}		(Basail et al., 2013)
Maçã	Liquid or stationary		MS+ BAP 7mg L^{-1}+ 1-Naphthaleneacetic acid (NAA) 1mg L^{-1}		(Lopes de Siqueira et al., 2013)
Musa acuminata × *Musa balbisiana*	Solid	MS + BAP 4mg L^{-1} + 1mg L^{-1} IAA	MS + BAP 4mg L^{-1} + 1mg L^{-1} IAA	MS + BAP 4mg L^{-1} + 1mg L^{-1} IAA	(Waman et al., 2015)
Rasthali AAB—Silk	Solid	MS + BAP 4mg L^{-1} + 0,2mg L^{-1}	MS + BAP 4mg L^{-1} + 0,2mg L^{-1}	MS + BAP 4mg L^{-1} + 0,2mg L^{-1}	(Uma et al.,2021)

According to Table 2, the differences found in the *in vitro* multiplication coefficient in the different banana varieties when exposed to different doses and hormonal relationships at different stages of explant development are presumed to be the product of genetic variability within a species; therefore, these responses depend on gene regulation mechanisms influencing shoot regeneration (Rios et al., 2013).

High levels of 6-benzylaminopurine (BAP) in the medium and its accumulation in explant tissues have been shown to inhibit shoot formation. Oliveira et al. (2016) found low proliferation of Maçã variety plantain shoots (1.54 shoots per explant) when using doses of 7 mg L^{-1} of BAP in the culture medium. Some researchers agree that the concentration of BAP should be around 2.5 to 5.0 mg L-1 (Lopes de Siqueira et al., 2013) during the initial and multiplication stages. Other authors report good results with concentrations of 0.5 and 1 mg L^{-1}. During the growth and development stages of the explant, different protocols have been tested in which the concentration of BAP is suppressed or reduced and/or complemented or replaced by different doses of auxins, especially by acid-3-indole acetic (3-IAA) or indole butyric acid (IBA) with the cytokinin/auxin ratio responsible for inducing proliferation of axillary buds.

According to Garcia et al. (2006), in cultivars with a high apical dominance, it is necessary to prepare several subcultures with high doses of BAP during the initial stage before passing the explants to the culture medium to obtain adventitious buds (García et al., 2006).When using the initial protocol proposed by Orellana et al. (2002) consisting of MS + BAP 4.0 mg L-1 + 3-IAA 0.65 mg L-1 and sucrose 30 g L-1 followed by MS medium supplemented with 1 mg L^{-1} of thidiazuron (TDZ) during the multiplication stages, it was shown to be possible to prevent apical dominance in the Gran Enano variety with a single subculture in the initial stage (25 days) and obtain high proliferation of adventitious buds in 60 days in the second subculture (Orellana et al., 2002). The dose of TDZ should not exceed a concentration of 1 mg L^{-1} since it has been shown that at higher doses, elongation and root formation in shoots are inhibited at higher concentrations.

The concentration and type of phytohormones in the formulation of culture media for the *in vitro* propagation of Musaceae on a large scale is variable and its adjustment is multifactorial. This type of formulation is necessary because the adjustment depends on the plant variety to be cultivated, the osmotic stress to which the plant tissues are subjected during explant disinfection, and the environmental conditions under which the culture is established (Waman & Bohra, 2019). When the indirect organogenesis pathway is chosen, the initial medium is supplemented with BAP and other cytokinins such as kinetin, zeatin, and TDZ, and with auxins, such as 3-IAA and IBA, which are subsequently replaced by variable doses of 2,4-dichlorophenoxyacetic acid (2,4-D) ranging between 1 and 5 mg L^{-1} to induce callus proliferation.

Phenolic Oxidation of Explants. The main cause of death of plantain explants planted *in vitro* is oxidation, which can be defined as the oxidation by free radicals of different cellular components and oxidation of phenolic

compounds catalyzed by the enzyme polyphenol oxidase (PPO) to produce quinones, which are highly reactive chemical species that can generate explant damage and lead to cell death (Azofeifa-Delgado, 2008).

Thus, phenolic oxidation causes tissue blackening due to the action of enzymes, such as PPOs and tyrosinases, that are released when tissues are injured; these enzymes cause oxidation of polyphenols and tyrosine into quinones that are phytotoxic, can polymerize, affect proteins, and consequently, inhibit the growth and viability of explants (Villegas et al., 2008).

Apart from explant darkening, oxidative stress has been related to the triggering of other physiological, morphological, epigenetic, and genetic disorders, such as recalcitrance, hyperhydricity, somaclonal variation, and habituation, that occur in cultured explants, (Azofeifa-Delgado, 2008). Oxidation in the case of plantains can lead to the death of cauline tips in the initial stages of development or interfere with performance during the multiplication phase (Camolesi et al., 2010). These researchers also proved that the production of phenoloxidase in plantain explants changes depending on plantain variety with some species being more susceptible than others to tissue phenolic oxidation.

It has been found that by planting plantain explants in a liquid medium in SIT temporary immersion systems (Villegas et al., 2008; Díaz & Castro, 2011; Pérez et al., 2013) and liquid media with or without agitation (Rodríguez et al., 2004; Ortega et al., 2010; Lopes de Siqueira et al., 2013), it is possible to prevent a large percentage of explant death due to oxidation. Frequent reseeding every 7 or 15 days for both stationary liquid media and semi-solid media, addition of activated carbon to the culture medium (Medina et al., 2015), initial growth of explants in the dark, initial growth of explants in medium, such as MS, that is diluted to half the concentration and/or devoid of Cu, Fe, and Zn (Azofeifa-Delgado, 2008) can help reduce the percentage of explant loss due to oxidation. In addition, a decrease in the concentration of the disinfectant solution and explant immersion times and treatment with antioxidant solutions are practices that can also help reduce oxidation-induced losses.

Environmental Factors. Light exposure has a direct influence on the development of explants. Cell differentiation and development under different conditions of light intensity and photoperiods appear to be genetically regulated. A photoperiod of 12 to 16 h with 1,000 to 3,000 lux was shown as sufficient to induce organogenesis (Litz & Jarrett, 1991) and has also been proven to be effective in different investigations carried out on banana micropropagation. The presence of light increases the accumulation of starch in cultured tissues, a process that is necessary for the formation of shoot primordia. Different photoperiods can also affect internal levels of growth regulators (Litz & Jarrett, 1991).

According to Azofeifa (2008), tissue oxidation can be reduced by obtaining the buds of mother plants that grow under conditions of low light intensity in the field and in the case of explants from *in vitro* plants, buds of etiolated plants that have been grown under dark conditions (Azofeifa-Delgado, 2008). This oxidation occurs because a high light intensity causes the amount of energy received by the chloroplast to be greater than that required for CO_2 fixation during photosynthesis; therefore, excess energy is captured by alternate electron acceptors and stimulates the formation of reactive oxygen species (ROS). Some research has shown that *in vitro* plantain cultures in the initial stage of explant establishment in the absence of light for periods ranging from 48 hours to 28 days (depending on the protocol) tissue oxidation is minimized (Florio et al., 1970; Sepúlveda et al., 2008). During the stages of differentiation, multiplication, and development, it has been found that photoperiods of between 12 and 16 h with light intensity between 30 and 68 µmol m^{-2} s^{-1} provide optimal conditions for *in vitro* plant regeneration. On the other hand, some researchers have found that the absence of light promotes callus formation, while its presence stimulates the development of axillary buds (Radice, 2010).

Temperature conditions depend on the species to be micro-propagated. In general, for the *in vitro* cultivation of plantain, most protocols report good results with temperatures between 24 and 30 °C, coinciding with the optimal temperatures for the development of these crops in the field.

On the other hand, relative humidity (RH) is directly related to dehydration of the explant; therefore, it is necessary to maintain the RH within the range between 80% and 90%. These percentages vary depending on the seal of the container so that in hermetically sealed containers, the RH approaches a value of 100%, while in containers with semi-permeable seals, the value can drop to 50% at which point the viability of the explant is compromised. Viability also depends on the conditions of the environmental humidity in the growth room. Notwithstanding all of

the above factors, very little research has been dedicated to establishing differences in the rates of multiplication and establishment of *in vitro* plantain plants by varying the environmental conditions of light, temperature, and RH and their relationships with the plantain genotype to be micro-propagated.

Currently, it has been suggested that success in the initiation of *in vitro* cultures of AAB Musaceae is directly associated with the explant season. Microbial interference can cause up to 90% mortality when initiation occurs during the rainy season, whereas the success rate increases during the drier months of the year (Josekutty et al., 2003; OS et al., 2010; Roy et al., 2010; Waman et al., 2015). The microbial load within the culture laboratory also varies with the season, resulting in concomitant increases in culture contamination during the rainy season (Patil et al., 2010). These results are consistent with the observations made at the CIAB of the UNAD in which only four seedlings successfully survived based on the contamination until reaching the phases of the primary and secondary establishment regardless of the disinfection protocol that was adapted for the establishment of the *in vitro* culture using 135 experimental corms of plantain. These corms and the *in vitro* establishment of the meristems were obtained in the municipality of Dosquebradas, Risaralda (4°49'49" N – 79°40'13" W) in which according to data from the Institute of Hydrology, Meteorology, and Environmental Studies (IDEAM), high volumes of precipitation are recorded, ranging between 2,000 and 2,500 mm per year, and the climate is humid (IDEAM, 2015).

Conclusion:-

In conclusion, the climate conditions under which the initial explant material is developed directly affect the susceptibility of the explant to damage due to completion and microbiological contamination since higher humidity conditions favor the microbiological load of the soil and consequently, the success of explant. In this sense, it can be said that the plant material obtained in the field in the municipality of Dosquebradas, Risaralda is not viable to be micro-propagated *in vitro* (Waman & Bohra, 2019). Thus, it is convenient to obtain the explant material from greenhouses under optimal conditions, which comply with the ICA regulations for plantain nurseries and guarantees a better-quality material. Additionally, genetic variability directly affects the response to the proliferation of axillary shoots in banana plants grown *in vitro* under different concentrations and ratios of growth regulators. Likewise, high proliferation and dissemination of the plantain disease, Moko, have established a need for reliable protocols for plantain micropropagation to provide farmers with certified planting material free of Moko and other diseases.

Conflict of interest:
The manuscript was prepared and reviewed by all the authors, who declare the absence of any conflict that could put the validity of the results at risk.

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