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Biological activity of biobras and Pectimorf-6 in each of the phases of micropropagation of banana (*Musa* sp.)

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ABSTRACT:

The use of biotechnology in the propagation of plantain and banana (*Musa* sp.) of great importance to induce, tolerant to plant genotypes for diseases and high yield potentials. However, auxins and cytokinins should be used, which are expensive and can sometimes cause changes in the regenerants obtained. Both traditional growth regulators (auxins and cytokinins) and non-traditional growth regulators (brassinosteroid analogues and mixtures oligogalacturonide) are used in the *in vitro* propagation of crops, but mush progress has been hindering due to the sufficient knowledge and impact of different phases prevailing in the micropropagation of banana hybrid 'FHIA-18' (AAAB) is present hitherto. This work was performed in order to evaluate the biological activity of an analogue of brassinosteroids (Biobras-6) [ABr] and a mixture of oligogalacturonide with the degree of polymerization between 9 and 16 (Pectimorf) [mOLG]. The effect of ABr and mOLG are determined as a substitute or complement of auxin (IBA or IAA) and cytokinin (6-BAP) for the establishment of *in vitro* multiplication and rooting of plantlets and in the acclimatization phase. Non-traditional regulators phenolization decrease the explant growth in the establishment phase of *in vitro* propagation; but increased the number of shoots per explants (above 3.5) and improved survival of vitro plant during the acclimatization phase.

Keywords:
Brassinosteroid analogues, *in vitro* and *ex vitro* culture oligopectatos

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INTRODUCTION

The growth and development is regulated by a group of chemicals called plant hormones (auxins, cytokinins, gibberellins, abscisic acid and ethylene) that interact with each other to meet the physiological needs of the plant. These hormones are synthesized in a given location of the plant and translocated to another, where they act at different concentrations (Gaspar *et al.,* 1996). The term growth regulator is more general and encompasses chemicals both natural and synthetic, that have similar biological effects to hormones, and influence the growth of plants from seed germination to senescence and are often used in the culture of *in vitro* plants (Kakani *et al.*, 2009; Amanullah *et al.,* 2010).

Traditional growth regulators (auxins, cytokinins and gibberellins) are used in different culture media for the mass propagation of plants, but other substances such as brassinosteroids are considered as the sixth class of plant hormones that are used at the concentrations up to 100 times lower in the culture media and exert a similar effect (Seeta *et al.,* 2002; Li *et al.,* 2005).

The oligogalacturonide also promote different morphogenetic, physiological and biochemical processes in plants, and are used in the culture media at different concentrations (1 to 15 or 0.47 to 7.05 $mg.L^{-1}$ μ^{-1} mol.L); similar range prescribed for traditional regulators, when expressed in the units of molar concentrations (10^{-7} -10^{-6} M) (Bellincampi *et al.,* 1995; Riou *et al.,* 2002; Simpson *et al.,* 2007). Both brassinosteroids and oligogalacturonide regulate, among other processes, the interaction between auxins, cytokinins, gibberellins and ethylene (Creelman and Mullet., 1997; Souter *et al.,* 2004), so that its introduction at a larger scale in a biotechnology process could make them more efficient.

Micropropagation, a commercial scale through the cultivation of meristematic apices (organogenesis) is a routine methodology widely used internationally (Kitto, 1997); It is a valuable technique in plants with poor production of propagules. In Cuba it is also frequently used in species that are spread over; such as banana (*Musa* sp.), which are of great importance for public consumption.

The cultivar 'FHIA-18', although mass produced *in vitro* propagated routinely in the Biofactories at Cuba; factors such as culture medium, dividing of explants, low coefficient of multiplication and the high percentage of phenolization, which could be higher or lower depending on the type of explant used to influence the decrease in the efficiency of productivity. Additionally, a chromosomal changes that can occur when subcultures are long and have low survival during acclimatization of plants. Furthermore, growth regulators could influence the phenotypic and genotypic changes, such as the presence of variegated plants (Pérez *et al.,* 2000; FHIA, 2007; Azofeifa, 2009).

The need for high quality materials for planting, has led to the search for new alternatives that ensure increased efficiency in the methods of *in vitro* propagation and acclimatization of the plants. Among these alternative cultivation, temporary immersion has helped in the short term, propagation of *in vitro* Bananas and plantains (*Musa* sp.) (Basail *et al.,* 2007). Also the long-term biological barriers that allow the efficient production of plants by somatic embryos in bioreactors expire (Escalona *et al.,* 2003) as the use of brassinosteroids and their analogs or oligogalacturonide (Diosdado, 1997; Barranco, 2002; Montes *et al.,* 2000). The aim of this study was to evaluate the biological activity of Biobras-6 and Pectimorf in each of the phases of micropropagation of banana (*Musa* sp.).

MATERIALS AND METHODS

The resistant black Sigatoka from Honduras, (FHIA, 2007): the hybrid banana (*Musa* sp.) 'FHIA-18' (AAAB group) was used for this study.

Homogenous individuals from mother plants having adequate performance, vigor, good physiological ability and health were transferred to controlled conditions in a grow house covered by a shade cloth made up (30%) polypropylene were selected. This is not a virgin that allows the passage of a density of photosynthetic photon fluxes of density 600 $\mu mol.m^{-2}\ s^{-1}$, that were measured with Light Meter Extech 401025 (United States). Subsequently, corns extracted (asexual seed) were removed until the outer parts of caulinar apex sections with diameter of 3 cm and 6 cm, approximately. The explants were washed with water and detergent and brushed on the surface to remove dirt. They subsequently underwent disinfection following the methodology proposed by Orellana *et al.* was followed. (2002).

Murashige and Skoog (1962) (MS) was supplemented with thiamine (0.30 $mol.L^{-1}m$), ascorbic acid (56.78 $\mu mol.L^{-1}$), myoinositol (15 $\mu mol.L^{-1)}$, sucrose (87.642 $\mu mol.L^{-1)}$ and agar (6.5 $gl^{-1)}$ as gelling agent (multiplication establishment phase and *in vitro* in the experiment) were used. The following growth regulators were used: 3-indole acetic acid (IAA), indole butyric acid, 3-(AIB), naphthalene acetic acid (NAA) and 6-benzylaminopurine (6-BAP). Moreover, a formulation of the brassinosteroid analogous spirits 25 (R) -2,3-dihydroxy-5-6-one- espirostan dihydroxy known as biobras- 6 or BB-6 (ABR) whose average molar mass is 446 $g.mol^{-1}$ and a mixture of 1,4-oligogalacturonide with a degree of polymerization between 9 and 16, known by the name of Pectimorf (mOLG), was used which has an average molar mass of 2042 $g.mol^{-1}$ (Cabrera *et al.,* 2003). The pH was adjusted to 5.7 ± 0.1, before sterilization and the mixture was autoclaved at 121^0 C and 1.2 $kg.cm^{-2}$ pressure.

The explants were cultured in the glass test tubes of size 2.5 x 9.0 cm (*in vitro* establishment phase) with a capacity of 250 mL (phases *in vitro* multiplication and rooting). In the *in vitro* establishment phase , 10 mL of culture medium was used and each explant was inoculated into the test tube; whereas in phases, *in vitro* multiplication and rooting medium 25 ml were added with 10 explants per culture. The containers with the explants were placed in the chamber under natural light at a temperature of 28 ± 1°C, relative humidity of 71% and a photosynthetic photon flux density of 65 $\mu mol.m^{-2}S^{-1}$ and used plants were with more than 4 cm and three developed leaves from the *in vitro* rooting phase, the same was performed immersing the roots in 0.1% Ridomil (commercial product Ridomil MZ 72% of systemic and contact) action except *vitro* plants that were treated with the ABr or mOLG. Planting was done in polystyrene trays containing 70 wells (125 cm^3) a substrate composed of a mixture of 75% by volume of rum decomposed over 25% of ferralitic red compacted. This phase was developed as reported by Pérez *et al.* (1999) and Anon (2003). Influence of growth regulators in the each phase of micropropagation of banana (*Musa* sp.) was studied.

Establishment phase *in vitro*

As shoots of banana plant material, which were inoculated in MS basal medium supplemented with IBA, 6-BAP, ABr or mOLG at different concentrations and the growth was monitered. The combinations that were used with ABr were: 1. MS alone (full control), 2. AIB (0.015$\mu mol.L^{-1)}$, 3. 6-BAP (17.77 $\mu mol.L^{-1)}$, 4. ABr (0.02 $\mu mol.L^{-1)}$ 5. ABr (0.1 $\mu mol.L^{-1)}$ 6. ABr (0.2 $\mu mol.L^{-1}$) 7. AIB (0.015 $\mu mol.L^{-1)}$ + 6-BAP (17.77 $\mu mol.L^{-1)}$.- control 8. 6-BAP (17.77 $\mu mol.L^{-1}$) + ABr (0.02 $\mu mol.L^{-1)}$, 9. 6-BAP (17.77 $\mu mol.L^{-1)}$+ ABr (0.1 $\mu mol.L^{-1)}$, 10. 6 BAP (17.77 $\mu mol.L^{-1)}$ + ABr (0.2 $\mu mol.L^{-1)}$, 11. AIB (0.015 $\mu mol.L^{-1)}$ + ABr (0.02 $\mu mol.L^{-1)}$,12. AIB (0.015 $\mu mol.L^{-1)}$ + ABr (0.1 $\mu mol.L^{-1)}$, 13. AIB (0,015 $\mu mol.L^{-1)}$ + ABr (0.2 $mol.L\ m^{-1)}$, 14. AIB (0.015 $\mu mol.L^{-1)}$ + 6-BAP (17.77 $\mu mol.L^{-1)}$ + ABr (0.02 $\mu mol.L^{-1)}$ 15. AIB (0,015 $\mu mol.L^{-1)}$ + 6-BAP (17.77 $\mu mol.L^{-1)}$ + ABr (0.1 $\mu mol.L^{-1)}$and 16. AIB (0.015 $\mu mol\ .L^{-1)}$ + 6-BAP (17.77 $\mu mol.L^{-1)}$ + ABr (0.2 $\mu mol.L^{-1)}$. The combinations

that were used with the mixture of oligogalacturonide (mOLG) were: 0.47; 2.35 µ and 4.70 mol.L^{-1} in the place of 0.02; 0.1 and 0.2 µmol.L ABr $^{-1}$, respectively. 30 explants were used per treatment and the percent survival and phenolization with the time it take to establish *in vitro* (emitting the first leaf) was evaluated.

Phase multiplication in *vitro*

Explants from the establishing medium [MS + IBA (0.015 µmol.L^{-1}) + 6-BAP (17.77 µmol.L^{-1}) .- control], underwent a longitudinal cut and placed in the MS basal culture medium with growth regulator of combinations described below: 1. MS only (complete control); 2. IAA (3.71 µmol.L^{-1}); 3. 6- BAP (17.77 µmol.L^{-1}); 4. ABr (0.02 µmol.L^{-1}); 5. ABr (0.1 µmol.L^{-1}); 6. ABr (mol.L 0.2 μ^{-1}); 7. IAA (3.71 µmol.L^{-1}) + 6-BAP (17.77 µmol.L^{-1})- control 8. 6-BAP (17.77 µmol.L^{-1})+ ABr (0.02 µmol.L^{-1}); 9. 6-BAP (17.77 µmol.L^{-1}) + ABr (0.1 µmol.L^{-1}); 10. 6-BAP (17.77 µmol.L^{-1}) + ABr (0.2 µmol.L^{-1}); 11. IAA (3.71 µmol.L^{-1}) + ABr (0.02 µmol.L^{-1}); 12. IAA (3.71 µmol.L^{-1}) + ABr (0.1 µmol.L^{-1}); 13. IAA (3.71 µmol.L^{-1}) + ABr (0.2 µmol.L^{-1}); 14. IAA (3.71 µmol.L^{-1}) + 6-BAP (17.77 µmol.L^{-1}) + ABr (0.02 µmol.L^{-1}); 15. IAA (3.71 µmol.L^{-1}) + 6-BAP (17.77 µmol.L^{-1}) + ABr (0.1 µmol.L^{-1}) and 16. IAA (3.71 µmol.L^{-1}) + 6-BAP (17.77 µmol.L^{-1}) + ABr (0.2 µmol.L^{-1}). The combinations used were similar to mOLG with the same concentrations and were used in the *in vitro* l establishment phase. The explants were cultured for four subcultures every 21 days and 50 explants were used per treatment. After each subculture the percentage of survival, the number of shoots, the height and the number of roots per explant were evaluated.

***In vitro* rooting phase**

Outbreaks of 1.5 cm from the treatment 7 [MS + IAA (3.71 µ mol.L^{-1}) + 6-BAP (17.77 µ mol.L^{-1}) .- control], were placed in the MS basal medium with growth regulators combinations as follows: 1. MS alone (full control), 2. IAA (7.42 µmol.$L^{-1)}$(control), 3. ABr (0.02 µmol.$L^{-1)}$ 4. ABr (0.1 µmol.$L^{-1)}$ 5. ABr (0.2 µmol.L $^{-1)}$ 6. IAA (mol.L 7.42 $\mu^{-1)}$ + ABr (0.02 µmol.$L^{-1)}$ 7. IAA (7.42 µmol.$L^{-1)}$ + ABr (0.1 µmol.$L^{-1)}$ 8. IAA (7.42 µmol.$L^{-1)}$ + ABr (0.2 µmol.$L^{-1)}$. For the experiment with mOLG treatments, 1 and 2 were similar to the experiment in ABR, other variants were: 3. mOLG (0.47 mol.L^{-1} m), 4. mOLG (2.35 µmol.$L^{-1)}$ 5. mOLG (4.70 µmol.$L^{-1)}$ 6. IAA (7.42 µmol.$L^{-1)}$ + mOLG (0.47 µmol.$L^{-1)}$ 7. IAA (7.42 µmol.$L^{-1)}$ + mOLG (2.35 µmol.$L^{-1)}$ and 8. IAA (7.42 m^{-1} mol.L) + mOLG (4.70 µmol.$L^{-1)}$. The explants were cultured for 15 days in the liquid culture medium. 100 plantlets were used per treatment with ABr and 50 plantlets per treatment for mOLG. Percent survival and rooting, number of leaves, height, number of roots and root length were evaluated.

Acclimatization phase

Plantlets were taken to the stage of *in vitro* rooting grown on MS + IAA (7.42 µmol.L^{-1}) Avg. First, dipping of roots were performed for 15 minutes in ABr or mOLG (I), 15 days after planting the plant is supplemented with ABr or mOLG (A) at 2 ml by *in vitro* plant and combination of both treatments (I + A) using a foliar spray. A control in which the immersion of the roots was carried out in 0.1% Ridomil and one with ANA; combinations with the same treatment described above was used. Treatments are shown in Tables 1 and 2. A total of 125 plantlets were used per treatment. After 60 days, when the end of acclimatization phase commensed survival percentage, height, number of leaves, pseudostem diameter, number and length of roots were evaluated.

The experiments were repeated twice and all the data for each of the indicators evaluated were collected using a completely randomized design and the data were processed by analysis of variance (ANOVA) for simple classification and the means were compared using Turkey test ($p \leq 0.05$). For data processing STATGRAPHICS statistical package was used

Table 1. Treatments used for the acclimatization of plantlets of banana Clone 'FHIA-18' (AAAB) with ABr

S.No	Treatments		Concentrations (µmol.L^{-1})	
	I	A	ANA	ABr
1	-	-	-	-
2	+	-	3.55	-
3	+	-	-	0.02
4	+	-	-	0.1
5	+	-	-	0.2
6	-	+	3.55	-
7	-	+	-	0.02
8	-	+	-	0.1
9	-	+	-	0.2
10	+	+	3.55	-
11	+	+	-	0.02
12	+	+	-	0.1
13	+	+	-	0.2

Bonus version 5.0 for Windows. Principal component analysis (PCA) (sections establishment and multiplication *in vitro* as well as in the acclimatization phase) was also performed using SPSS version 11.5 for Windows. The formation of groups was performed according to the spatial distribution of treatments in the graph, according to the investigator's discretion. The formed groups underwent a discriminant analysis to test the effectiveness of the groups performed. In all the cases, previously normal distribution (Kolmogorov-Smirnov) and homogeneity of variance (Bartlett) were checked.

RESULTS AND DISCUSSION

Influence of growth regulators in the each phase of micropropagation of banana (*Musa* sp).

Establishment phase *in vitro*

From the biplot representation corresponding to Principal Component Analysis (PCA) (Figure 1) a differential behavior between treatments were evaluated *in vitro,* in the establishment phase with the use of brassinosteroid analogue (ABr) was observed. The first two components (C_I and C_{II}) accounted for 98.23% of variability. Four groups were identified, which were applied for discriminant analysis, which confirmed that 97.01% of the groups were classified legetimately. In this sense, explants treatment 8 (6-BAP (17.77 µmol.L^{-1}) + ABr (0.02 µmol.L^{-1})] formed the group I (subgroup 1), which had the lowest concentration of analog which reached higher survival (93.30%), lower phenolization (22.90%) and the same in 19 days. Also, the explants of treatment 11 also formed (subgroup 2) group I since survival was 84.80%, the phenolization of 31.45% and explants were set at 20 days. The explants of both treatments are in the same group, differed among themselves and reached above explants that were established in seven treatments (group II and 1 group IV), under absolute control, for monitoring results respectively.

To evaluate the effect of oligogalacturonide (mOLG) mix in the *in vitro* establishment of the explants as shown in Figure 2; corresponding to the ACP treatments performed for BIPLOT that was represented were evaluated. The C components C I and C II accounted for 97.35% of variability. Six groups were identified, which were applied for discriminant analysis,

Table 2. Treatments used for the acclimatization of plantlets of banana Clone 'FHIA-18' (AAAB) with mOLG

S.No	Treatments		Concentration (µmol.L^{-1})	
	I	A	ANA	mOLG
1	-	-	-	-
2	+	-	3.55	-
3	+	-	-	0.47
4	+	-	-	2.35
5	+	-	-	4.70
6	-	+	3.55	-
7	-	+	-	0.47
8	-	+	-	2.35
9	-	+	-	4.70
10	+	+	3.55	-
11	+	+	-	0.47
12	+	+	-	2.35
13	+	+	-	4.70

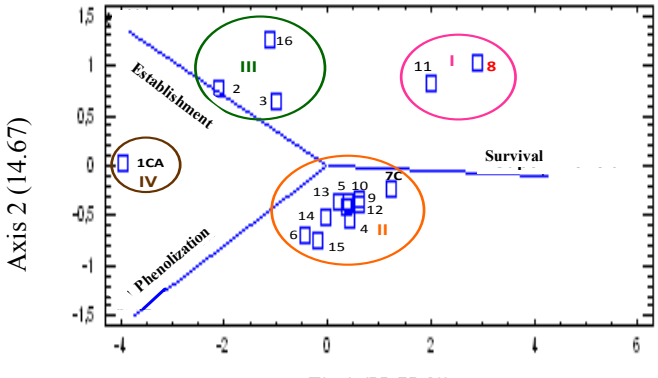

Figure 1. Biplot representation of the results of the different treatments that were evaluated in the phase of *in vitro* establishment of banana (*Musa* sp.) Clone'FHIA-18' (AAAB) by principal component analysis (n = 60) (1.- Absolute control , 2.- AIB (0.015 µmol.L-1), 3.- 6-BAP (17.77 µmol.L-1), 4.- ABr (0.02 µmol.L-1), 5.- ABr (0.1 µmol.L-1), 6.- ABr (0.2 µmol.L-1), 7.- AIB (0.015 µmol.L-1) + 6-BAP (17.77 µmol.L- 1) .- control, 8.- 6-BAP (17.77 µmol.L-1) + ABr (0.02 µmol.L-1), 9.- 6-BAP (17.77 µmol.L-1)) + ABr (0.1 µmol.L-1), 10.- 6-BAP (17.77 µmol.L-1) + ABr (0.2 µmol.L-1), 11.- AIB (0.015 µmol.L-1) + ABr (0.02 µmol.L-1), 12.- AIB (0.015 µmol.L-1) + ABr (0.1 µmol.L-1), 13.- AIB (0.015 µmol.L-1) + ABr (0.2 µmol.L-1), 14.- AIB (0.015 µmol.L-1) + 6-BAP (17.77 µmol.L-1) + ABr (0.02 µmol.L-1), 15.- AIB (0.015 µmol.L-1) + 6-BAP (17.77 µmol.L-1) + ABr (0.1 µmol.L-1), 16.- AIB (0.015 µmol.L-1) + 6-BAP (17.77 µmol.L-1) + ABr (0.2 µmol.L-1)

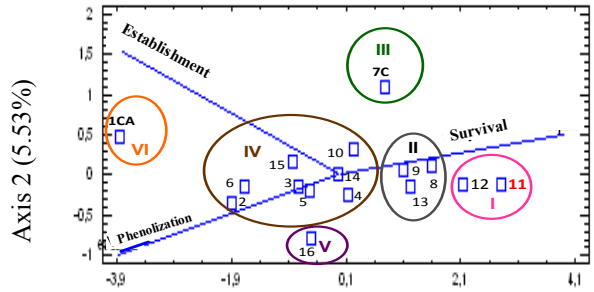

Figure 2. Biplot representation of the results of the different treatments that were evaluated in the phase of *in vitro* establishment of banana (*Musa* sp.) Clone'FHIA-18' (AAAB) by principal component analysis (n = 60) (1.- Absolute control , 2.- AIB (0.015 µmol.L-1), 3.- 6-BAAIB (0.015 µmol.L-1) + 6-BAP (17.77 µmol.L- 1) .- control, 8.- 6-BAP (17.77 µmol.L-1) + mOLG (0.47 µmol.L-1), 9.- 6-BAP (17.77 µmol.L-1)) + mOLG (2.35 µmol.L-1), 10.- 6-BAP (17.77 µmol.L-1) + mOLG (4.70 µmol.L-1), 11.- AIB (0.015 µmol.L-1) + mOLG (0.47 µmol.L-1), 12.- AIB (0.015 µmol.L-1) + mOLG (2.35 µmol.L-1), 13.- AIB (0.015 µmol.L-1) + mOLG (4.70 µmol.L-1), 14.- AIB (0.015 µmol.L-1) + 6-BAP (17.77 µmol.L-1) + mOLG (0.47 µmol.L-1), 15.- AIB (0.015 µmol.L-1) + 6-BAP (17.77 µmol.L-1) + mOLG (2.35 µmol.L-1), 16.- AIB (0.015 µmol.L-1) + 6-BAP (17.77 µmol.L-1) + mOLG (4.70 µmolP (17.77 µmol.L-1), 4.- mOLG (0.47 µmol.L-1), 5.- mOLG (2.35 µmol.L-1), 6.- mOLG (4.70 µmol.L-1), 7.- .L^{-1})

which confirmed that 95.85% of the groups were correctly classified.

The best results were obtained when integrally replaced in the culture medium 6-BAP by mOLG. Therefore, treatment explants 11 [AIB (0.015 µmol.L^{-1}) + mOLG (0.47 µmol.L^{-1}) (subgroup 1), reached a 93.35% survival and 33 phenolization , 00%, better than that of control [1 (group VI) and 7 (group III)] treatment, and settled *in vitro* meristematic apices for 20 days, before the corresponding treatments of 1 and 7 explants starts, that is, the absolute control and control, respectively. Likewise, treatment 12 [AIB (0.015 µ

mol.L^{-1}) + mOLG (2.35 µmol.L^{-1})], which also ranked in group I (subgroup 2), obtained a phenolization (37.70%) and days to settle delayed in explants *in vitro* (21 days) similar to the treatment 11, but survival was observed to be low (86.70%). Explants with seven treatments (control) and 1 (full control) were located in two separate groups, III and VI respectively, lower than previous treatments of explants in all the variables that were assessed for values.

From the above results it can be inferred, that it is not sufficient to use the basal culture medium without growth regulators for the establishment of the explants banana clone FHIA-18 '(treatment 1) group IV (Figure 1) and group V (Figure 2), and that the factors relating to the organogenic response is age, physiological characteristics and the stage of development of the donor plant or tissues used as a source of explants (Zhang and Lemaux, 2004).

Although age in the same in all explants, there are differences in the content of endogenous hormones, amino and tissue absorption capacity thereof; aspects that were not studied in this thesis but which could be addressed in future research. The report was found only at Saucedo *et al.* (2008), malanga (*Xanthosoma sagittifolium* (L.) Schott.), shows that they have achieved a 68.75% survival in the explants in MS medium without growth regulators.

The establishment of explants was found to be 81.50% with phenolization 53.33%: If a auxin alone (treatment 2) Group III (Figure 1) and group IV (Figure 2), was used results were generally lower (survival is employed 28 days) but when the MS basal medium is supplemented with ABr (treatments 4, 5 and 6) (survival: 64.83 to 76.70%; phenolization: 55.45 to 66.65% and establishment of explants: 20-21 days) (group II, Figure 1) or mOLG (treatments 4, 5 and 6), (survival: 46.63 to 66.68%; phenolization: 51.05 to 64.60% and establishment explants: 24-26 days) (group IV, Figure 2), there may be an excess of auxin in the culture medium which did not favor the survival of the explants and slowed the time required for the same were established *in vitro*. 64.98% Phenolization of 59.30% was established with the cytokinin alone treatment (6-BAP) (treatment 3) (Group III or Group IV Figure 1, Figure 2), was done the results were higher (survival was used explants 26 days) when the auxin alone (AIB) (treatment 2, Figures 1 and 2) was used as survival was highest and lowest phenolization occurred, so a better relationship between cytokinin and auxin could be established for the better growth of explants.

Another group prefer the combination of an auxin (IAA, IBA or NAA) and cytokinin (6-BAP) to promote the establishment of explants (Ascough *et al.,* 2009). Such is the case of Zaffari *et al.* (2000), who reported that the most suitable culture medium for the establishment phase in the banana clone 'Grand Nain' (AAA) was the MS supplemented with IAA (5.71 µmol.L^{-1}) and 6-BAP (4.44 µmol.L^{-1}), but the explants were not ready to be sectioned and transferred to the multiplication medium until 60 days Uzcátegui *et al.* (2010) reported that the best behavior *in vitro* during this phase of establishment of the apexes of plantain (*Musa* AAB) cv. 'Harton' and 'Hartón Double Stem' was when the MS basal medium was supplemented with 5 mg.L^{-1} 6-BAP (22.21 µmol.L^{-1}) and 1.30 mg.L^{-1} of AIB (0.065 µmol.L^{-1}), that necrosis of the explants was 25% and 80% survival.

In this phase a substitute should be used for AIB ABr and the substitute is mOLG for 6-BAP. There are also different results regarding the use of ABr in the culture media for the establishment of plantain and banana (*Musa* sp.), as these analogs have the potential to replace 6-BAP. Barranco reported similar results (2002), as 0.01 mg.L^{-1} Biobras-6 (0.02 µmol.L^{-1}) replaced 6-BAP and achieved 81.9% germination of somatic embryos of banana clone 'FHIA-18' and Diaz *et al.* (2004) achieved the best results with plantain when the AIB was replaced by 0.05 mg.L^{-1} Biobras-6 (0.1 µmol.$^{-1}$) since

improved survival of explants (90%).

The oligogalacturonide is also used in the different biotechnological processes (Diosdado, 1997; Suarez and Hernández, 2008). However, there are few reports on the usage of establishment of the explants. Diaz *et al.* (2004), argued that Pectimorf 5 mg.l^{-1} (2.35 μmol.L^{-1}) can be used as a substitute for 6-BAP plantain. These results coincide with those of this work, but the mOLG (Pectimorf) was used at a lower concentration (0.47 μmol.L^{-1}) equal to 1 mg.L^{-1} Montes *et al.* (2000), also achieved very good survival of explants in *Anthurium cubense* L., when they used a 10-fold higher concentration of Pectimorf (4.70 mM).

Also, phenolic oxidation was not a direct inhibitory factor of survival in the *in vitro* establishment of explants that reared with ABr or mOLG. This aspect was also observed by Villegas *et al.* (2008) where oxidation did not affect the formation of somatic embryos from the immature male flowers of cultivar 'Grand Nain' (AAA).

From the results of this first phase of *in vitro* culture for propagation of hybrid 'FHIA-18', it is suggested to use ABr (0.02 μmol.L^{-1}) or mOLG (0.47 ⌈mol. L $^{-1}$) as substitutes of auxin (AIB) in the first case and cytokinin (6-BAP), in the second, in a MS basal medium.

Phase multiplication in vitro

The survival of explants that were obtained during the multiplication *in vitro* with respect to ABr and mOLG was high; regardless of the treatment used and subculture, it ranged from 90.00 to 100%. When the culture medium was supplemented with 6-BAP (17.77 μmol.L^{-1}) and ABR (0.1 μmol.L^{-1}) (Treatment 9), the greatest number of shoots per explant was reached and outbreak of treatment of 3, 7, 8, 9, and 10 does not showed rooting (Table 3). The mOLG 0.47 μmol.L^{-1}) combined with 6-BAP (17.77 μmol.L^{-1}) (treatment 8) produced 9.65 shoots per explant in total (1 subculture is: 2.06, 2 do subculture: 2.26; is 3 subculture: 2.33; subculture to 4: 3.00) and outbreaks of such treatment and 3 and 7 not rooted in any of the four subculture treatments and rooted to 9 from the third subculture (Table 3).

The phase of *in vitro* multiplication is very important in any micropropagation protocol, as this is where the number of shoots per explant increases. However, it is a very complex phase where the results vary among species and cultivars. Usually survival is high, unless high concentrations of growth regulators are prolonged resulting in death of subculture explants that are used for phenolic oxidation (Poulose *et al.,* 2007).

The above results showed that phase without regulators promotes growth and rooting of explants, but the number of outbreaks, which is the fundamental objective of this phase, since they apparently did not have enough endogenous hormones that would allow cell division; Similar results are presented only with IAA, which also favored the desired process.

Currently, some commercial micropropagation laboratories grow plantain and banana (*Musa* sp.) Using only 6-BAP; However, this may vary depending on the cultivar, the explant source, and the type and concentration of growth regulator that is used. In this regard, Shirani *et al.* (2009) reported that banana genotypes of Berangan Intan 'Berangan,' Rastali ',' Nangka 'and' Baka Baling of the MS basal medium, supplemented with 6-BAP (22.2 to 44.4 mM) increased the number of shoots per explant, but higher than the 33.3 uM concentrations caused abnormalities.

The ABr only behaved similarly to the IAA, and the need for the use of IAA and 6-BAP is evident for the existence of a higher shoot formation. Similar results were reported by Jiménez *et al.* (2004) for the clone banana 'FHIA-21', which reached a multiplication rate of 3.8 and issued no roots when the explants were inoculated into semisolid MS medium supplemented with 4 mg. L $^{-1}$ 6-BAP (17.77 μmol.L^{-1}) and 0.05 mg.L $^{-1}$ Biobras-6 (0.1 μmol.L^{-1}). Generally, when an increase

Table 3. Number of shoots and roots per explant (n = 100) of banana (Musa sp.) Clone 'FHIA-18' (AAAB) with the use of ABr (A) and MOLG (B) for four subcultures the multiplication phase

N^0	Concentrations (mmol.L^{-1})				Number of Outbreaks				Number of Roots			
	IAA	6-BAP	ABr	mOLG	1	2	3	4	1	2	3	4
ABr							**A**					
1CA	-	-	-	-	1.10^{e}	1.06^{e}	1.05^{f}	1.00^{d}	1.55^{b}	1.43^{bc}	1.25^{b}	1.15^{e}
2	3.71	-	-	-	1.30^{d}	1.40^{cd}	1.25^{e}	1.10^{d}	1.20^{c}	1.30^{cd}	2.05^{a}	2.10^{abc}
3	-	17.77	-	-	1.50^{c}	1.60^{c}	1.70^{c}	1.85^{bc}	0^{d}	0^{e}	0^{c}	0^{f}
4	-	-	0.02	-	1.40^{cd}	1.50^{cd}	1.50^{de}	1.30^{cd}	1.25^{bc}	1.15^{cd}	1.30^{b}	2.10^{abc}
5	-	-	0.1	-	1.35^{bc}	1.40^{cd}	1.35^{e}	1.20^{d}	1.30^{bc}	1.20^{cd}	1.40^{b}	2.25^{ab}
6	-	-	0.2	-	1.25^{de}	1.25^{d}	1.20^{e}	1.10^{d}	2.00^{a}	1.85^{ab}	1.95^{a}	2.35^{ab}
7C	3.71	17.77	-	-	1.73^{b}	1.80^{b}	1.93^{b}	2.33^{b}	0^{d}	0^{e}	0^{c}	0^{f}
8	-	17.77	0.02	-	1.35^{d}	1.50^{cd}	1.65^{c}	2.00^{b}	0^{d}	0^{e}	0^{c}	0^{f}
9	-	17.77	0.1	-	2.00^{a}	2.15^{a}	2.40^{a}	2.95^{a}	0^{d}	0^{e}	0^{c}	0^{f}
10	-	17.77	0.2	-	1.30^{d}	1.45^{cd}	1.55^{de}	2.05^{b}	0^{d}	0^{e}	0^{c}	0^{f}
11	3.71	-	0.02	-	1.25^{d}	1.50^{cd}	1.70^{c}	1.35^{cd}	1.00^{c}	0.90^{d}	1.10^{b}	2.00^{abcd}
12	3.71	-	0.1	-	1.30^{d}	1.50^{cd}	1.60^{c}	1.25^{d}	1.20^{c}	1.10^{cd}	1.25^{b}	2.15^{abc}
13	3.71	-	0.2	-	1.25^{de}	1.45^{cd}	1.70^{c}	1.20^{d}	2.10^{a}	2.00^{a}	2.15^{a}	2.60^{a}
14	3.71	17.77	0.02	-	1.20^{de}	1.25^{d}	1.40^{de}	1.50^{bcd}	2.00^{a}	1.30^{cd}	1.20^{b}	1.40^{de}
15	3.71	17.77	0.1	-	1.15^{e}	1.25^{d}	1.30^{e}	1.40^{cd}	2.00^{a}	1.40^{c}	1.35^{b}	1.50^{cde}
		EE			0.09**	0.10**	0.12***	0.17***	0.07***	0.08**	0.09**	0.03*
			mOLG								**B**	
1CA	-	-	-	-	1.10^{de}	1.06^{d}	1.05^{e}	1.00^{f}	1.55^{bcd}	1.43^{c}	1,25^{de}	1.15^{c}
2	3.71	-	-	-	1.30^{cd}	1.40^{cd}	1.25^{de}	1.10^{ef}	1.20^{c}	1.30^{bc}	2.05^{bc}	2.10^{b}
3	-	17.77	-	-	1.50^{bcd}	1.60^{bc}	1.70^{bc}	1.85^{bcd}	. 0^{d}	0^{d}	0^{f}	0^{d}
4	-	-	-	0.47	1.20^{cd}	1.40^{cd}	1.53^{cd}	1.46^{def}	1.73^{ab}	1.80^{ab}	2.13^{ab}	3.13^{a}
5	-	-	-	2.35	1.26^{cd}	1.53^{bc}	1.60^{bc}	153^{cdef}	1.80^{ab}	1.93^{a}	2.20^{ab}	3.00^{a}
6	-	-	-	4.70	1.06^{d}	1.20^{cd}	1.33^{cd}	1.40^{def}	1.80^{ab}	1.93^{a}	2.53^{a}	3.46^{a}
7C	3.71	17.77	-	-	1.73^{b}	1.80^{b}	1.93^{b}	2.33^{b}	0^{d}	0^{d}	0^{f}	0^{d}
8	-	17.77	-	0.47	2.06^{a}	2.26^{a}	2.33^{a}	3.00^{a}	0^{d}	0^{d}	0^{f}	0^{d}
9	-	17.77	-	2.35	1.66^{bc}	1.73^{b}	1.80^{bc}	2.06^{bc}	0^{d}	0^{d}	0.80^{e}	1.06^{c}
10	-	17.77	-	4.70	1.53^{bc}	1.66^{bc}	1.73^{bc}	1.86^{bcd}	0.20^{d}	0.40^{d}	0.93^{e}	1.13^{c}
11	3.71	.	-	0.47	1.46^{cd}	1.53^{bc}	1.66^{bc}	1.86^{bcd}	1.13^{c}	1.33^{bc}	1.46^{bcd}	1.93^{b}
12	3.71	-	-	2.35	1.33^{cd}	1.46^{cd}	1.53^{cd}	1.80^{bcd}	1.86^{ab}	1.93^{a}	1.93^{bc}	2.00^{b}
13	3.71	-	-	4.70	1.20^{cd}	133^{cd}	1.40^{cd}	1.60^{cde}	213^{a}	2.26^{a}	2.33^{ab}	2.40^{b}
14	3.71	17.77	-	0.47	1.26^{cd}	1.33^{cd}	1.33^{cd}	1.53^{cdef}	1.,93^{ab}	2.00^{a}	2.00^{ab}	2.20^{b}
15	3.71	17.77	-	2.35	1.13^{de}	1.26^{cd}	1.26^{de}	1.46^{def}	1.93^{ab}	2.13^{a}	2.20^{ab}	2.33^{b}
		EEx			0.11**	0.12*	0.12**	0.11*	0.10**	0.09***	0.10**	0.10***

IAA.- 3-indoleacetic acid; 6-BAP.- 6-benzylaminopurine; mOLG.- mixture of oligogalacturonides; 1,2,3,4.- number of subcultures; C.-control; n.- total explants of the experiment in the two repetitions; CA.- absolute control; EEx.- Standard error of the mean; Averages with different letters differ statistically according to the Tukey test ($p < 0.05$) (* significant for $p < 0.1$; ** significant for $p < 0.01$; *** significant for $p < 0.001$)

in height is limited the formation of new shoots occurs and nutrients and reserves of the explants is used in the elongation and no cell division.

The results presented here and others (Diosdado, 1997; Rodriguez, 1999;. Nunez *et al.,* 2005) showed that the analogue of brassinosteroids (ABR) have similar biological activity *in vitro* natural brassinosteroids, despite the structural changes that occur in the side chain, which can be combined with traditional growth regulators to promote cell division and differentiation.

When mOLG (0.47 μmol.L^{-1}) was used as a substitute for IAA (3.71 μmol.L^{-1}) (treatment 8), the number of outbreaks increased from first (2.06) to fourth in the subculture (3.00). In the subcultures, the shooting and height of the shoots were similar to the control treatment sprouts. It seems that the brassinosteroid analogue, mixed oligogalacturonide actively involved in the cell division, by increasing the number of outbreaks when used in conjunction with 6-BAP. One of the first reports that the Pectimorf used in biotechnology, was in the protoplast culture of sour orange (*Citrus aurantium* L.) (Diosdado, 1997), achieving best results with the concentration of 10 mg.L^{-1}. Subsequently, Montes *et al.* (2000) studied the response of leaf explants *in vitro* plants of *Anthurium cubense* L. and found that the best results were achieved with 4.7 uM Pectimorf. They obtained a regeneration rate up to 17 buds from the callus which had been cultured *in vitro*.

Apparently, interaction between the mOLG and 6 -BAP were added to the culture media and hormones present in the explants were established, since, cell differentiation was favored, compared to the medium that is supplemented with IAA and 6-BAP. It should be noted at this stage that both mOLG and ABr as in any concentration were able to replace the IAA at concentrations of 0.1 and 4.70 $^{-1}$ μmol.L stand, respectively, for values obtained with all indicators statistically higher than the control treatment.

***In vitro* rooting phase**

The plantlets were obtained with ABr ranged from 91.00% to 100% and from 90.65% to 100% rooting and those obtained with mOLG, these variables ranged from 97.00 to 100% and 95, 45-100%, respectively. 100% survival and rooting was obtained when the ABr (0.1μmol.L^{-1}) or mOLG (4.70 μmol.L^{-1}) was used as substitutes for IAA.

From the biplot representation for the ACP (Figure 3) a differential behavior between treatments were evaluated in the *in vitro* rooting phase with the use of brassinosteroid analogue (ABr). The C components I and C II accounted for 83.34% of variability. Five groups were identified, which were applied for discriminant analysis, which confirmed that 95.50% of the groups were correctly classified. *Vitro* plants of Treatment 4 [ABr (0.1 μmol.L^{-1})] (Group I) showed the best results regarding the number of shoots (5,75), roots (4.05) and height (4.66 cm), while the root length (4.71 cm) were higher than the treatment 7 [IAA (7.42 μmol.L^{-1})) + ABR (0.1 μmol.L^{-1})] (5.02 cm) (group II) and 6 [IAA (7.42 μmol.L^{-1}) + ABr (0.02 μmol.L^{-1})] (4.88 cm) (Group IV). The group III was integrated with the *vitro* plants of treatments 1 (MS only) and 2 [IAA (7.42 μmol.L^{-1})], control and absolute control, respectively with the lower values for length of roots (4.05 and 4.12 cm, respectively) and among the lowest in terms of height (4.29 and 4.25 cm, respectively) and number of shoots (4.70 and 4.45, respectively).

Vitro plants treatments 6 [IAA (7.42 μmol.L^{-1}) + ABr (0.02 μmol.L^{-1})] (group IV) and 8 [IAA (7.42 μmol.L $^{-1}$) ABr (0.2 μmol.L^{-1})] (group V) formed two separate groups, with lower values 2.70 for height 4.14 and 4.13 cm, and number of roots 2.82, respectively, and among the lowest in the number of shoots with respective values of 4.55 and 4.20.

Results to evaluate the effect of mixing oligogalacturonide (mOLG) *in vitro* rooting explants are shown in Figure 4; and the same corresponding to the

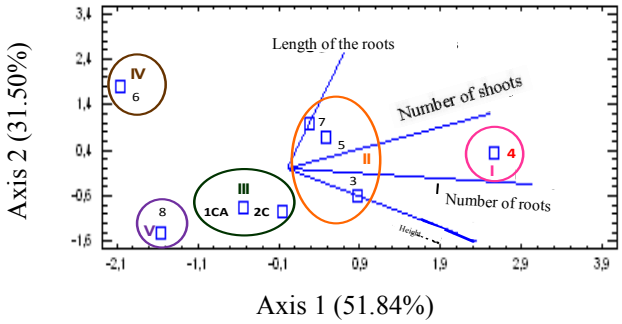

Figure 3. Biplot representation of the results of the different treatments that were evaluated in the in vitro rooting phase of the banana (Musa sp.) Clone 'FHIA-18' (AAAB) by principal component analysis (n = 200) (1.- MS only .- Absolute control, 2.- IAA (7.42 µmol.L^{-1}) .- control, 3.- ABr (0.02 µmol.L^{-1}), 4.- ABr (0.1 µmol.L^{-1}), 5.- ABr (0.2 µmol.L^{-1}), 6.- IAA (7.42 µmol.L^{-1}) + ABr (0.02 µmol.L^{-1}), 7.- IAA (7.42 µmol.L^{-1}) + ABr (0.1 µmol.L^{-1}) and 8.- IAA (7.42µmol.L^{-1}).

ACP treatments was performed th results were calculated with BIPLOT. The components C I and C II accounted for 95.36% of variability. Five groups were identified, which were applied for discriminant analysis, which confirmed that the plantlets were obtained with mOLG (4.70 µmol.L^{-1}) (treatment 5) and showed the best results in terms of number of leaves (5.70), height (4.89 cm) and number of roots (4.60) and formed a separate group (I); was placed below the treatment 4 [mOLG(2.35 µmol.L^{-1})] shoots with 4.5, 4.60 and 4 cm roots, with intermediate values as to the length of the roots (4.38 cm) (group II). Group III showed intermediate values for the number of shoots (4.50 to 4.70), root height (4.25 to 4.41 cm) and length (4.05 to 4.17 cm).

The group was made up of *in vitro* plants IV treatments 6 [IAA (7.42 µmol.L^{-1}) + mOLG (0.47 µmol.L^{-1})] and 7 [IAA (7.42 µmol.L^{-1}) + mOLG (2.35 µmol.L^{-1})], which had one of the lowest values in the number of leaves (4.10 and 4.05, respectively) and height (4.22 and 4 , 10 cm, respectively), the number of roots reached intermediate values (4.00 and 3.5, respectively) and the best results in root length (5.27 and 5.02 cm, respectively form). The latter group, V, was formed by vitro plants treatment 8 [IAA (7.42 µmol.L^{-}

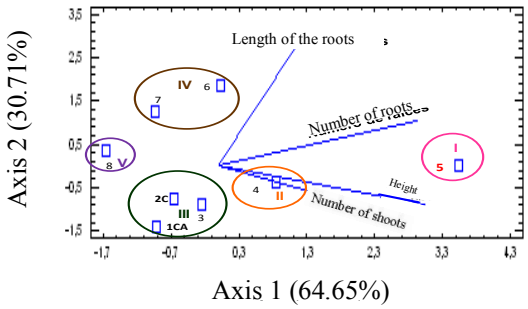

Figure 4. Biplot representation of the results of the different treatments that were evaluated in the in vitro rooting phase of the banana (Musa sp.) Clone 'FHIA-18' (AAAB) by principal component analysis (n = 100) (1.- MS only . - Absolute control, 2.- IAA (7.42 µmol.L^{-1}) .- control, 3.- mOLG (0.47 µmol.L^{-1}), 4.- mOLG (2.35 µmol.L^{-1}), 5.- mOLG (4.70 µmol.L^{-1}), 6.- IAA (7.42 µmol.L^{-1}) + mOLG (0.47 µmol.L^{-1}), 7.- IAA (7.42 µmol.L^{-1}) + mOLG (2.35 µmol.L^{-1}) and 8.- IAA (7.42 µmol.L^{-1}) + mOLG (4.70 µmol.L^{-1})

^{1}) + mOLG (4.70 µmol.L^{-1})] with the lowest values in the number of shoots (4,00) and height (4.00 cm), between the lowest in the number of roots (3.30) and intermediates in root length (4.52 cm). Five groups, to which was applied a discriminant analysis and 98.55% of them were well formed (Figure 4).

The root development in plants is determined by the characteristic patterns for each species, which in turn depends on the interaction with the other components of the culture medium and the interactions between hormones of *vitro* plant and growth regulators which are added exogenously in the medium. The number of roots that develop a plan is genetically determined, however, their number varies depending on the culture medium and environmental conditions. In this research, an increasing trend was observed thereof when the plantlets were exposed to different concentrations of ABr or mOLG. Similar results were reported by Nieves *et al.* (2006) and Hector *et al.* (2007).

Tsun-Thai *et al.* (2005) succeeded in rooting of banana clones Berangan and 'Mas' in MS basal medium supplemented with 5m M of IAA. Meanwhile, Rodriguez (1999) reported that *in vitro* plants of banana clone 'Grand Nain' reached a greater number of roots when MS medium was supplemented with 1.3 mg.L^{-1} IAA (7.42 µmol.L^{-1}) and 0.05 mg.L^{-1} Biobras-6 (0.1 µmol.L^{-1}). However, the root length was greater when IAA was replaced by Biobras-6 at concentrations of 0.01 mgL^{-1} (0.02 µmol.L^{-1}) and 0.05 mg. L^{1} (0.1 µmol.L^{-1}).

According Bellincampi *et al.* (1993), a mixture of oligogalacturonide with a degree of polymerization between 8 and 16, inhibited the formation of roots in snuff explants (*Nicotiana tabacum* L.), and the GUS gene was inhibited with auxin activity. The results of this study shows, the observed increase in the number of roots with mOLG in banana hybrid 'FHIA-18'. Results that support this work are the Falcon and Cabrera (2007) African Violet (*Saintpaulia ionantha* H. Wendl), by which Pectimorf forwards the rooting process compared to the IAA.

Moreover, Jimenez *et al.* (2004) when they replaced the IAA by Biobras-6 in a hybrid 'FHIA-21', also found that increased in the number of roots of plantlets.

Contrary to what was reported by Bellincampi *et al.* (1993, 1995 and 1996), the oligogalacturonide inhibit root formation in snuff (*Nicotiana tabacum* L.) and bean (*Phaseolus vulgaris* L.). Other authors such as Rodríguez (1999) achieved a greater number and root length when they used Pectimorf 10 mg.L^{-1} (4.70 µmol.L^{-1}).

The increase in this indicator *in vitro* conditions with ABr (0.1 µmol.L^{-1}) and mOLG (4.70 µmol.L^{-1}) could be due, among other reasons, to a response by plantlets to maintain the absorption capacity of water and mineral salts, which could affect the length reduction, as evidenced by the inverse relationship, which typically is established between the number of roots which emit them and their growth in length. This is best seen in *in vitro* conditions, because the roots at this stage are not functional to 100% and moreover, a larger number of roots in vitro plants assures their attachment to the culture medium and are the two elements essential for the good growth and development.

The comprehensive analysis of the results achieved at this stage of *in vitro* rooting allows recommend the use of ABr (0.1 µmol.L^{-1}) or mOLG (4.70 µmol.L^{-1}) as substitutes to AIA, although in the latter case to stimulate this process was necessary to use 10 times more product than in the previous stages.

Acclimatization phase

The acclimatization phase is essential in any micropropagation protocol, since the main purpose of it is to achieve high survival of plantlets after hardening. From the biplot representation for the ACP (Figure 5) held for treatments and variables that were evaluated in the acclimatization phase with the use of brassinosteroid analogue (ABr), a differential behavior between

modalities were as well as concentrations of the analog. The components C I and C II accounted for 92.81% of variability. Three groups were identified, which were applied discriminant analysis, which confirmed that 95.74% of the groups were correctly classified.

In group I the plants that underwent root immersion for 15 minutes and the foliar spraying for 15 days after planting with different concentrations of the ABR (I + A) [13 treatments (subgroup 1); 11 and 12 (subgroup 2) and which underwent foliar spraying 15 days after planting (A) (Treatment 9) and that was performed immersing the roots 15 minutes before planting (I) (treatment 5. This is formed after the last two treatments of subgroup 3.

The best results were obtained for plants with treatment 13 [I + A (Apr.- mol.L 0.2 m^{-1})], with survival (96.65%), plant height (7.15 cm), number of leaves (7.10), pseudostem diameter (8.73 mm), number of roots (7.20) and root length (7.60 cm). The results of the treatments 11 [I + A. ABr (0.02 µmol.L^{-1})] and 12 [I + A (Apr.- 0.1 µmol.L^{-1})] decreased than treatment 13 showing survival values of 90.40 %; the height of the plant ranged from 6.98 to 7.16 cm; leaf number between 5.50 to 6.00; pseudostem diameter was greater than 8.40 mm and the number of roots ranged from 5.70 to 6.00; plants treatments 5 [I. ABr (0.2 µmol.L^{-1})] and 9 [A. ABr (0.2 µmol.L^{-1})] also formed group I, but with lower values in all the variables with respect to previous treatments were evaluated.

The group II is with very heterogeneous treatment of plants where 2 controls are present [I. ANA (3.55 µmol.L^{-1})], 6 [A. ANA (3.55 µmol.L^{-1})were] and 10 [I + A ANA (3.55 µmol.L^{-1})], Other treatments of the plant along with the immersion of the roots for 15 minutes (I) or foliar spray has made on the plants 15 days after planting (A). The group III of formed plants were obtained treatment 1 (absolute control) with the lowest scores on all variables evaluated: survival (84.65%), plant height (5.80 cm) number of shoots (4.60 cm), pseudostem diameter (7.73 mm), number of roots (4.85) and root length (4.91 cm).

From the biplot representation for the ACP (Figure 6) held for treatments and variables that were evaluated in the acclimatization phase with the use of mixed oligogalacturonide (mOLG), a differential behavior between modes was observed due to application of oligogalacturonide concentrations. The C components I and C II accounted for 95.74% of variability. Four groups were identified, which were applied discriminant analysis, which confirmed that 96.38% of the groups were correctly classified.

Generally, the immersion treatment of the roots and foliar spray of the plants 15 days after planting (I + A) was greater than the roots dipping (I) or spraying the plants (A). In Group I plants underwent dipping roots and for 15 days were sprayed with mOLG, treatments 11 [I + A (mOLG.- mol.L 0.47 μ^{-1})] (subgroup 1) and 12 [I + A (2.35 µmOLG.- mol.L^{-1})], and also underwent treatment, 3 [I (mOLG.- 0. 47 µmol.L^{-1})] and 7 [A (mOLG.- mol.L 0.47 μ^{-1})], the latter three treatments formed the subgroup 2. Plants of the best treatment 11, reached the survival of (96.75%), a height (7.08 cm), the number of shoots (7,00), pseudostem diameter (8.63 mm), the number of roots (7.45) and length of the roots (7.90 cm).

Group II was composed of three control treatments (2, 6 and 10) and treatment 8 [A. mOLG (2.35 µmol.L^{-1})]. The group III that was intermediate in the same plants were grouped with treatments of 4, 5, 9 and 13. The fourth group formed plants of treatment 1 (absolute control), usually those with the lowest values in the variables were evaluated: survival (84.65%), height (5.80 cm), number of leaves (4, 60), pseudostem diameter (7.73 mm), number of roots (4.85) and root length (4.91 cm.)

Low photosynthetic activity of *in vitro* is one of the major factors affecting the efficiency of micropropagation and therefore influences its low

survival in this phase (Teixeira *et al.,* 2005). In *Tuberaria major* (Willk.), Goncalves *et al.* (2010), achieved a 97% survival of six weeks after transplantation and Jiménez *et al.* (2004), achieved greater plant height in clone 'FHIA-21' by combining, dipping the roots in a solution of ANA (10 $mg.L^{-1)}$ and Biobras-6 (0.05 $mg.L^{-1}$) during the acclimatization phase.

In general, dipping roots combined with the foliar spray of ABr or mOLG, apparently *in vitro* plant as, improved processes of absorption, transport of water and nutrients in the *in vitro* plants and increased the photosynthetic activity, which favored its growth during acclimatization. In addition, both brassinosteroids and their analogs, such as oligogalacturonide increased the plant height, number of leaves, pseudostem diameter, number and length of roots and decreased stress induced by the techniques of *in vitro* culture. These results were similar to the previous results reported in different cultures (Montes *et al.,* 2000; González-Olmedo *et al.,* 2005; Choe, 2006; Bajguz and Hayat, 2009), is shown by the results obtained in this stage, both the ABR ($mol.L^{-1}$ 0.2 μ) as well as the mOLG (0.47 $\mu mol.L^{-1}$) can replace the auxin (NAA) and the combination was more effective due to immersion of the roots and subsequent plant foliar spray.

CONCLUSION

- Biobras-6 (ABR) in combination with 6-benzylaminopurine (6-BAP) was superior to the combination of auxin-cytokinin (6-BAP and AIB-IAA-6-BAP) established in each of the phases. Mass micropropagation of banana (*Musa* sp.) clone 'FHIA-18' (AAAB) and the Pectimorf (mOLG) was seen, except for *in vitro* establishment phase where it can replace the cytokinin.
- The immersion of the roots for 15 minutes and foliar spray or vitro plants banana (*Musa* sp.) 15 days after planting with ABr or mOLG favored the survival and growth of plants, modifying the leaf anatomy, which decreased the stressful effects associated with the transfer of the conditions *in vitro* to ex vitro (acclimatization).

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