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EFFECT OF THE PECTIMORF® IN MERISTEM TIP *In Vitro* CULTURE CASSAVA (*Manihot esculenta* Crantz), CLONE´CMC-40´ AND ´SEÑORITA´

Efecto del Pectimorf® en el cultivo de ápices de plantas *in vitro* de yuca (*Manihot esculenta* Crantz), clones `CMC-40´ y `Señorita´

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ABSTRACT. The development of more efficient and more sustainable methodologies in the material obtaining yucca (*Manihot esculenta* Crantz) *in vitro*, the improvement of the quality of the seed and vegetable material sanitation, are the objectives of this work to evaluate the effectiveness of Pectimorf® (mixture of oligogalacturonides), innocuous and natural substance taken place in Cuba, to be used as possible complement or substitute of the growth regulators used traditionally in the propagating medium of yucca meristematics tip. It was demonstrated that Pectimorf® in the means of cultivation, possible to establish *in vitro* of the meristem tip cultivation in yucca clones ´CMC-40´ and ´Señorita´ and it stimulated the explant growth too. The results contribute to understand the mechanisms of action of this substance and their future application *in vitro* plant resorts in Cuba.

Key words: micropropagation, oligosaccharides, cultivation medium, meristems

RESUMEN. El desarrollo de metodologías más eficientes y sostenibles en la obtención de material *in vitro* de yuca (*Manihot esculenta* Crantz), favorece al mejoramiento de la calidad de la semilla y el saneamiento del material vegetal, por ello se trazó como objetivo evaluar la efectividad del Pectimorf® (mezcla de oligogalacturónidos), sustancia inocua y natural producida en Cuba, a emplearse como posible complemento o sustituto de los reguladores del crecimiento empleados tradicionalmente en el medio de cultivo para el crecimiento de ápices meristemáticos de yuca. Se demostró que el Pectimorf® en el medio de cultivo, posibilitó el establecimiento *in vitro* de los ápices en clones de yuca ´CMC-40´ y ´Señorita´ y favoreció el crecimiento de los explantes. Los resultados contribuyen al esclarecimiento de los mecanismos de acción de esta sustancia y su aplicación futura en las unidades de propagación masiva de plantas del país.

Palabras clave: micropropagación, oligosacáridos, medio de cultivo, meristemas

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is a very versatile crop planted by small growers in more than 1000 countries (1), since it has the facility, as food, of being dehydrated and preserved for some years (2). Cassava world production in 2012 reached 282 million tonnes, equivalent to a 7 % increase compared to the volume reached in 2011. The cassava outlook leads to an expanded production since it continues to be a strategic crop for food security and poverty relief (1).

In Cuba, this crop is a valuable food since ancient times and is part of the roots and tubers supply named by Cubans as “viandas”. It is an important component of the traditional food basket of the population; besides, as animal food where the cassava foliage is also used in protein plots for livestock feeding and in the production of concentrates to feed pigs and poultry. According to the Ministry of Agriculture (3), the strategic projection (2010- 2015) predicted an increased planted area and yields for coming years. In order to meet this challenge, it is essential to increase the seed volumes demanded by Cuban growers.

Within the national varietal strategy defined by the National Research Institute on Tropical Roots and Tubers (INIVIT), the clones ‘CMC-40’ and ‘Señorita’ are present. The first one has the feature of being a

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short-cycle clone (from 6 to 10 months) with a high productivity, while clone ´Señorita´ is a short-cycle one, over 10 months, but stands out for its excelent culinary quality.

This crop is vegetatively propagated and its multiplication is usually tedious and slow (4). Cuban farmers have perpetuated this tuber by using asexual seeds (cuttings or stem pieces) in repeated plantings which is a risk for the possibility of spreading out pests[A]. The production of high quality planting material is a basic need for our growers to expand the crop.

The low availability of certified cassava material (*Manihot esculenta* C.) in the country is similar to what happens to the rest of the agamic reproduction crops and it could be solved by applying *in vitro* propagation techniques. According to different authors[B] (5, 6), *in vitro* techniques provide advantages like recovery of the vigor and productivity of the plants. Likewise, it can contribute to the production of high-quality seed volumes to propagate virus and pathogen free plants.

The development of efficient and quick methods of cassava plant regeneration with *in vitro* culture, either by somatic embryogenesis[A] (7, 8, 9) or organogenesis[C,D] (10), are generally demanding as per the composition of the culture medium, particularly the use of growth regulators, so it would be important to achieve the use of local bioactive products for the *in vitro* propagation of cassava (*Manihot esculenta* C.), as an alternative to improve the effectiveness of the process. Pectimorf® is one these bioactive products, it is manufactured by the Department of Plant Physiology and Biochemistry, from the National Institute of Agricultural Sciences (INCA), Mayabeque, Cuba[E].

Pectimorf® is a mixture of α-1,4-olygogalacturonides with polymerization degree (GP) from 9 to 16 (11). It is considered a powerful elicitor of defense in plants (12, 13), growth stimulant and cell differentiation in several plant species[E] (14).

The response to this mixture of olygogalacturonides (OG), whose effect is similar to auxins' or cytokinins', can be mainly due to the hormonal balance of the explant and the composition of growth regulators in the culture medium; moreover, they regulate the interaction between auxins, cytokinins, gibberelins and ethylene, among others, which backs up the use of this substance as a promising alternative for Cuba's plant biotechnology (15, 16).

However, Pectimorf® effect over cassava (*Manihot esculenta* C.) meristematic tips cultured *in vitro* remains unknown for the clones ´CMC-40´ and ´Señorita´. It is also unknown the possible partial or total replacement of ANA (naftaleneacetic acid) and 6-BAP (6- bencylaminopurine), as the growth regulators traditionally used in culture media.

MATERIALS AND METHODS

The trial was conducted at the Biotechnology Laboratory of the Genetic and Plant Breeding Department of the National Institute of Agricultural Sciences (INCA), located at the municipality of San José de Las Lajas, Mayabeque province.

Plant material

Two clones from the Cuban germplasm collection of cassava, pertaining to the Research Institute of Tropical Roots and Tubers (INIVIT), Santo Domingo, Villa Clara province, with contrasting characteristics as to the cycle, productivity and other morphoagronomic characters.

Characteristics of the clone ´CMC-40´

Semi-erect growing plants, with more than 2 branches. Dark brown stems, young green-reddish color foliage, red petioles, adult leaves, young pink leaves, leaves with 5-7 simple lobes, upward irregularly inclined petioles. More than 10 roots per plant, rough surface and oblique growth, conic or cylindrical sesils, outer film of dark beige color, pink bark and white pulp. Among their main features, its high productivy and short cycle (6 to 10 months) stand out.

[A] Medero, V. *Embriogénesis somática en yuca* (*Manihot esculenta* Crantz) [Tesis de Doctorado], Universidad de Ciego de Ávila, 2006.

[B] Garcia, G.; Magaly, V. y Rodríguez Morales, S. ''Effect of meristem culture micropropagation on the vigor and yield of the cassava clone «Señorita».'' [en línea], (eds. Roca, W.M. y Thro, A.M.), *Proceedings of the First International Scientific Meeting of the Cassava Biotechnology Network*, edit. Centro International de Agricultura Tropical, Cartagena, Colombia, 1993, [Consultado: 8 de septiembre de 2015], Disponible en: <http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=catalco.ormato=2&cantidad=1&expresion=mfn=007899>.

[C] Dawit, B. *Micropropagation of Selected Cassava Varieties* (*Manihot esculenta* Crantz) *from Meristem Culture* [Master Science Thesis], Addis-Abeba University, 2009, 36 p.

[D] Medero, V.; Rodríguez, S.; Borroto, C.; Gómez, R.; López, J.; de Feria, M.; García, M.; Ventura, J.; del Sol, L.; Cabrera, M.; Pons, C.; Cortés, C.; Martínez, M.; Álvarez, M. y García, J. *Sistema de inmersión temporal para producción intensiva de material de siembra de yuca*, [3], Continente yuquero. Informativo del Consorcio Latinoamericano y del Caribe de Apoyo a la investigación y Desarrollo de la Yuca (CLAYUCA), 2001, pp. 10-11.

[E] Izquierdo, H. *Empleo de nuevas sustancias como reguladores del crecimiento en la micropropagación del banano (Musa spp) clon «FHIA-18» (AAAB)* [Tesis de Doctorado], Instituto Nacional de Ciencias Agrícolas, Mayabeque, Cuba, 2013, 87 p.

Chacteristics of the clone ´Señorita´

Erect-growing plants, with no branches or little branches. Very vigorous stem and short internodes. Green yellow stem with yellow-pink buds, green leaves with slightly pink midribs in adult leaves, in younger leaves petioles are red on the upper side and green-red on the lower side. Short white roots, each plant produces an average of 8-12, rather superficial which facilitates harvest. The cycle is long, more than 10 months. This clone stands out for its excelent culinary quality.

Cuttings of 15-20 cm long from adult plants of the above-mentioned clones were taken and planted on pots of 14 cm of upper diameter and a height of 10 cm, containing substrate made up of a mixture of 25 % organic matter (decomposed filterpress mud) and 75 % of compressed red ferralitic soil (17), at a rate of 1: 2 v/v. Shoots grew up on a metal structure screenhouse with aphid-proof screen walls, nylon roof covered by a black shadow cloth of polypropilene (30 %) and cement floor. Irrigation was practiced periodically (twice a week), in order to maintain the substrate humid above 85 %.

Four weeks after planting, shoots from the cuttings were cut and were divided into three sections of 3 to 5 cm long with two buds and they were taken to the lab.

Preparation and disinfection of explants

Explants were disinfected according to the methodology proposed by Medero[A]. At the flow table, meristematic tips of 0,5-0,7 mm long were extracted with the aid of a stereoscopic microscope CARLZEISS with a magnification of 4X. Later on, meristematic tips were introduced into test tubes (25 X 150 mm) with 20 mL of solid medium. The basal medium MS (Murashige & Skoog, 1962) (18), plus thiamine (1 mg L^{-1}), mioinositol (100 mg.L^{-1}), sucrose (20 mg L^{-1}) and agar (6,5 mg L^{-1}) as gellifying agent, were also used. After 21 days, the tip of the *in vitro* plants (0,8-1,0 mm long) was extracted by using the stereoscopic microscope CARLZEISS with a magnification of 4X. A total of 15 explants per treatment was used for each cultivar.

Differente Pectimorf® concentrations were used as substitute and complement of growth regulators ANA (naftaleneacetic acid) and 6-BAP (6-bencylaminopurine) used in the control medium for the growth and development of meristematic cassava tips. In all cases, gibberelic acid (AG_3) was maintained to facilitate the elongation or node segments. Table I shows the characteristics of each treatment.

Growth regulators

- Naftaleaneacetic acid (ANA): Reagent from Merck
- Gibberelic acid (AG_3): Reagent from Merck
- 6 bencylaminopurine (6-BAP): Reagent from Merck
- Pectimorf®: From the National Institute of Agricultural Sciences (INCA). Laboratory of Plant Physiology and Biochemistry, Mayabeque, Cuba. Natural and healthy product, made up of a mixture of biologically active polysacharids produced from a citrus pectine whose active principle is a mixture of α-1,4-oligogalacturonides polymerization degree (GP) from 9 to 16.

Culture conditions

Sterilization was done with an autoclave for 20 minutes at 121 ^{0}C with 1.5 atmospheres. Plant material was placed in culture chambers at 25 ± 2°C, relative huimidity 80-90 % and artificial light with a photoperiod of 16 hours light, and a light intensity of 18,75 µmol m^{-2} s^{-1}.

Table I. Treatments used to establish cassava tips *in vitro* (*Manihot esculenta* C.), clones ´CMC-40´ and ´Señorita´

Treatments	ANA (mg L^{-1})	AG_3 (mg L^{-1})	BAP (mg L^{-1})	Pectimorf® (mg L^{-1})
1 (control)	0,02	0,05	0,04	-
2	-	0,05	-	-
3	-	0,05	0,04	5
4	-	0,05	0,04	10
5	-	0,05	0,04	15
6	0,02	0,05	-	5
7	0,02	0,05	-	10
8	0,02	0,05	-	15
9	0,02	0,05	0,04	5
10	0,02	0,05	0,04	10
11	0,02	0,05	0,04	15
12	-	0,05	-	5
13	-	0,05	-	10
14	-	0,05	-	15

The pH of the culture media was adjusted to 5.7 in all cases, before the addition of Agar.

After 21 days, the following observations were made: height (cm): it was measured with a rule from the stem base till the top of the foliage; number of leaves: leaves per vitroplant were individually quantified; number of roots: the total number of roots per vitroplant was quantified; shoots color: it was determined with the aid of a hexadecimal color code[F].

A totally randomized design was used with 10 explants per treatment, trials were repeated twice in time and data from the observations were statistically processed by a simple classification analysis of variance (ANOVA totally randomized) and the means were compared according to Duncan's ($p \leq 0,05$). Data processing used the software package SPSS for Windows (19). In all cases, the normal distribution and homogeneity of the variance were checked.

RESULTS AND DISCUSSION

During the first week of establishment, some alterations in the color and morphology of the apices were observed in the treatments where Pectimorf® was used, presumably due to the increase of the speed of growth or cell division in this first phase. In spite of this, the apices presented a lime green coloration (32CD32) and from the remaining days continued their growth normally.

As shown in Table II there were significant differences among treatments with a differentiated response of the variables in both clones. As to vitroplants height, clone ´CMC-40´, reached the maximum height 2,88 cm with treatment 3; however, it did not statistically differ from the control, from treatments 6 and 7 where Pectimorf® was added in the presence of ANA. The results indicate that the product could compensate the effect of the auxin (ANA) when it was absent in the medium; however, there was not a marked depressive effect (antagonic) when it was present**.**

In the clone ´Señorita´ maximum height was recorded in treatments 6 and 7, where Pectimorf® was used in the presence of ANA and AG_3 at concentrations of 5 and 10 mg L^{-1}, which did not statistically differ among them reaching values above 2 cm height; both treatments did not differ from the control, treatment 3 (6-BAP + 5 mg L^{-1} Pectimorf®) and from the treatment where Pectimorf® was used in the presence of ANA at the concentration of 15 mg L^{-1}. In this case, it was necessary the presence of ANA + Pectimorf®, and the product had a similar effect to cytokinins by filling the absence of BAP and stimulating cassava tips growth.

The effect of cytokinins on the growth and *in vitro* morphogenesis has been shown to be important because if the relationship between auxin / cytokinin on the culture medium is low, it favors callus formation and viceversa, if it is high, it favors rooting (20, 21).

For this character, it can be said that treatment 3, (ANA 5 mg L^{-1} of Pectimorf®) was able to promote meristems growth in ´CMC-40´ without differing from others; however, the clone ´Señorita´ required the presence of the auxin and concentrations of 5 and 10 mg L^{-1} of Pectimorf®, treatments[B] (5), which leads to think that the endogenous content of auxins is lower so an exogenous addition is needed to achieve the appropriate balance for tips growth.

As to the number of leaves in clone ´CMC-40´, when Pectimorf® was used at the concentration of 15 mg L^{-1} in the presence of both regulators, vitroplants reached 5,5 leaves without statistical differences from treatment 3, but it did with the rest of the treatments. The treatment that recorded the lowest values was treatment 2, that pertains to the culture medium without growth regulators ANA and 6-BAP. In treatment 3, developed leaves had a green forest color (228B22), which did not ocurred in treatment 11, since they were yellow (FFFF00). It is very probable that a hormonal unbalance has taken place originating changes in the synthesis of pigments related to chlorophyll.

In the clone ´Señorita´, maximum leaf number values were recorded in treatments 6, 7 and 14, which did not statistically differ among them, nor with the rest of the treatments, except treatment 2 that pertains to the medium without growth regulators (ANA, BAP) and without Pectimorf, in which leaves showed a yellow color (FFFF00) also present in treatmernts 9, 10 y 11. In the rest of the treatments, leaves were spring green (00FF7F). This clone seems to have a lower level of endogenous auxins, sop their absence in the culture medium favored that Pectimorf® concentrations caused a positive effect to induce an increased cell division of the buds originating leaves.

In the clone ´CMC- 40´ the best treatment for the number of roots was 3, containing 6-BAP and 5 mg L^{-1} of Pectimorf®, without significant differentes with the control and with treatments 6, 7, 9, 10 and 11 that contained ANA and 5 and 10 mg L^{-1} of Pectimorf® respectively, in the case of the first two and ANA and BAP with 5, 10 and 15 mg L^{-1} of Pectimorf® for the last three. It seems this indicator did not depend on the exogenous concentrations to cause differences in the number of roots which could be justified for the origin and mobilization of auxins in root formation.

[F] *Nombres de colores hexadecimales* [en línea], [Consultado: 20 de septiembre de 2015], Disponible en: <http://www.disfrutalasmatematicas.com/numeros/hexadecimales-colores-nombres.html>.

Table II. Pectimorf® effect on the morphological variables *in vitro* cassava tips (*Manihot esculenta* C.), clones ´CMC-40´ and ´Señorita´ cultured during 21 days (n=20)

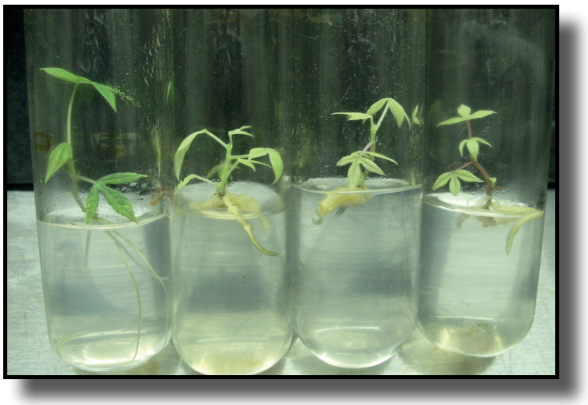
Treatments	CMC- 40				Señorita			
	Height (cm)	Number of sheets	Number of roots	Color	Height (cm)	Number of sheets	Number of roots	Color
1 (Control)	2,00 ab	2,8 bc	2,8 abc	Forest green (228B22)	1,12 ab	2,0 ab	1,8 ab	Forest green (228B22)
2	0,76 b	1,66 c	1,4 bc	yellow (FFFF00)	0,42 b	1,0 b	0,2 d	yellow (FFFF00)
3	2,88 a	4,0 ab	4,75 a	Forest green (228B22)	1,34 ab	3,2 ab	3,2 ab	Forest green (228B22)
4	0,77 b	2,0 c	1,80 bc	Forest green (228B22)	0,6 b	2,2 ab	1,0 bc	green lime (32CD32)
5	0,85 b	2,0 c	0,75 bc	Forest green (228B22)	0,4 b	1,8 ab	0,4 cd	green lime (32CD32)
6	2,12 ab	2,60 bc	3,6 ab	green lime (32CD32)	2,08 a	3,6 a	2,4 ab	green lime (32CD32)
7	2,37 ab	2,75 bc	3.5 ab	yellow green (ADFF2F)	2,18 a	3,6 a	4,0 a	green lime (32CD32)
8	1,02 ab	2,5 bc	1,5 bc	yellow green (ADFF2F)	1,10 ab	2,6 ab	3,4 ab	green lime (32CD32)
9	1,20 ab	4,0 ab	3,0 abc	yellow (FFFF00)	0,8 b	2,6 ab	3,0 ab	yellow (FFFF00)
10	1,35 ab	4,0 ab	3,5 ab	yellow (FFFF00)	0,64 b	3,0 ab	2,2 ab	yellow (FFFF00)
11	1,17 ab	5,5 a	2,0 abc	yellow (FFFF00)	0,72 b	2,0 ab	1,6 ab	yellow (FFFF00)
12	0,72 b	2,0 c	1,0 bc	Forest green (228B22)	0,66 b	1,6 ab	2,0 ab	Forest green (228B22)
13	1,02 ab	2,25 bc	1,2 bc	Forest green (228B22)	0,76 b	1,8 ab	1,8 ab	Forest green (228B22)
14	1,22 ab	1,75 c	0,4 c	Forest green (228B22)	0,56 b	3,4 a	2,4 ab	Forest green (228B22)
ES	0,41*	0,40**	0,59**		0,43*	0,46**	0,58***	

Treatment 1: ANA+ AG3+ BAP, treatment 2: without regulators (ANA; BAP)+ AG3 , treatment 3: AG3+ BAP+ 5 mgL^{-1} Pectimorf®, treatment 4: AG3+ BAP+ 10 mgL^{-1} Pectimorf®, treatment 5: AG3+ BAP+ 15 mgL^{-1} Pectimorf®, treatment 6: ANA+AG3+5 mgL^{-1} Pectimorf®, treatment 7: ANA+AG3+10 mgL^{-1} Pectimorf®, treatment 8: ANA+AG3+ 15 mgL^{-1} Pectimorf®, treatment 9: ANA+AG3+ BAP+5mgL^{-1} Pectimorf®, treatment 10: ANA+AG3+ BAP+ 10 mgL^{-1} Pectimorf®, treatment 11: ANA+AG3+BAP+15 mgL^{-1} Pectimorf®), treatment 12: AG3+5mgL^{-1} Pectimorf®, treatment 13: AG3+10mgL^{-1} Pectimorf® y treatment 14: AG3+15mgL^{-1} Pectimorf®) Means with different letters are statistically different according to Duncan's test ($p \leq 0,05$)(* significative for $p<0,1$; **significative for $p<0,01$; ***significative for $p<0,001$).

As to the number of roots for the clone ´Señorita´, the highest number was recorded for treatment 7, with ANA and 10 $mg.L^{-1}$ of Pectimorf®, without significant differences with the treatments containing 0,02 $mg.L^{-1}$ of ANA at all Pectimorf® concentrations, nor with the control treatment in the presence of ANA and BAP and of treatments 12, 13 and 14 without ANA and BAP with the rest of Pectimorf® concentrations. The tested concentrations of this product greatly contributed to the mobilization of necessary auxins for an adequate root system development.

Figure 2 shows thickened roots, maybe due to the hormonal unbalance between auxins and cytokinins which could produce an excess in the endogenous levels of the explant by interacting with AG_3 so causing thickned roots in both clones. It did not happen in the rest of the treatments.

Rhizogenesis *in vitro* is a complex process that needs to be looked at deeper since several factors influence, namely, the composition of the culture medium and the initial explant (specie, genotype and physiological age).



The image is significant for the clone ´Señorita´. (from left to right, control treatment, treatment 9: 0,02 mg L^{-1} ANA+0,05 mg L^{-1} AG3+ 0,04 mg L^{-1} 6-BAP+ 5 mg L^{-1} Pectimorf®, treatment 10: 0,02 mg L^{-1} ANA+0,05 mg L^{-1} AG3+ 0,04 mg L^{-1} 6-BAP+ 10 mg L^{-1} Pectimorf® and treatment 11: 0,02 mg L^{-1} ANA+0,05 mg L^{-1} AG3+ 0,04 mgL^{-1} 6-BAP+ 15 mg L^{-1} Pectimorf®)

Figure 1. Pectimorf® effect as complement of ANA and 6-BAP on the growth of cassava tips (*Manihot esculenta* C.), clone CMC-40 after 21 days of culture

The image is significant for the clone ´Señorita´. (from left to right control treatment, treatment 2: growth regulators free ANA and 6-BAP, except 0,05 mg L^{-1} AG_3, treatment 12: 5 mg L^{-1} Pectimorf®, treatment 13: 10 mg L^{-1} Pectimorf® and treatment 14: 15 mg L^{-1} Pectimorf®)

Figure 2. Pectimorf® effect as total hormonal substitute of auxins and cytokinin on the growth of cassava tips (*Manihot esculenta* C.) cv ´CMC-40´ of 21 days of culture

Treatments using Pectimorf® as total substitute of regulators ANA andy BAP[D] (7, 10) recorded the worst results with clone ´CMC-40´, however, the absence of those regulators affected to a lesser degree, the clone ´Señorita´, since these treatments reached means higher than the clone ´CMC-40´, mainly at concentrations of 10 and 15 mg L^{-1} of Pectimorf® and the color was forest green (228B22) (Figure 2). This figure shows yellow leaves (FFFF00) for both clones in treatment 2, pertaining to the medium free of growth regulators ANA and 6-BAP and without Pectimorf. It seems that the presence of AG_3 is not enough to activate physiological mechanisms promoting a response in the formation of some leaf pigments and in shoots height. Gibberelin action is mainly directed to internode growth, to the removal of dormancy in buds and seeds, to induce and accelerate flowering and participate in tuberization (22, 23).

However, other authors (24) confirmed a synergic effect of Pectimorf® with AIA, that influenced the increased germination percentage of artificial seed capsules of sugarcane without reaction in the olygosacharid-gibberelin interaction, though it was in the treatment where Pectimorf® was combined with both growth regulators (AIA and GA_3) that the highest number of plants was achieved.

In cassava crop (*Manihot esculenta* C.) there are many authors using the auxin naftaleneacetic acid (ANA) as growth regulator in the micropropagation protocols, and also the cytokinin bencylaminopurine (6-BAP), to achieve a higher efficiency of the process (9, 10).

Olygogalacturonides are also used in different biotechnological processes as: cassava seedling growth (*Manihot esculenta* C.) (24), embryo culture of *Citrus macrophylla* W[G], sprout culture of white dasheen (*Colocassia* spp) (25), formation of embryogenic callus of mandarin ´Cleopatra´ (*Citrus reshni* Hort. ex Tan) (16) and meristematic tips of banana (*Musa* spp.)[E].

Results showed a similar Pectimorf® effect to that of auxins over the growth of cassava buds in both cultivars, because there were not damages or reduction in the absence of ANA; however, other authors say that olygogalacturonides have a marked antiauxinic effect (26, 27), which could occur because the composition of Pectimorf® is a mixture of olygogalacturonides with a variable polymerization degree.

On the other hand, several research works confirm that Pectimorf® has a similar effect to auxins' for the *in vitro* establishment of sweet potato buds (*Ipomea batata* L) (28), mainly by those who used Pectimorf® (10 mg L^{-1}) to replace indol-3-acetic acid (0,05 mg L^{-1}) and gibberelic acid (10 mg L^{-1}), making possible bud growth with all Pectimorf®.concentrations.

[G] Bao, L. *Efecto del Pectimorf y diferentes brasinosteroides en la embriogénesis somática de Citrus macrophylla* Wester [Tesis de Grado], Universidad de La Habana, La Habana, Cuba, 2009, 57 p.

In sugarcane (*Sacharum* spp.), at the hystodifferentiation stage of callus, the highest number of embryos and the highest synchrony took place in those combinations where 2,4-D was reduced in half the concentration of 5 mg L^{-1} of the olygosacharid (29).

In the *in vitro* propagation of *Spathiphyllum* spp (14) the treatment consisting in the reduction of the 6-BAP to half the concentration to use in the control medium (0,5 mg L^{-1}) and the addition of 10 mg L^{-1} of Pectimorf® produced the highest quantity of shoots in the plants.

In the cultivar ´CMC-40´ the inclusion of Pectimorf® at the lowest concentration (5 mg L^{-1}) to replace ANA (0,02 mg L^{-1}), combined with cytokinin (6-BAP) resulted in a superior height, number of leaves and roots, though without differences in the control; however, clone ´Señorita´, required the presence of ANA and concentrations of 5 and 10 mg L^{-1} of Pectimorf® to reach the best results as to height, number of leaves and roots; even these treatments influenced leaf color enough to promote the formation of pigments in them; taking into account that both treatments did not show significant differences in this clone, so 5 mg L^{-1} of Pectimorf® with 0,02 mg L^{-1} ANA and without BAP could have been used. This difference in the behavior of Pectimorf® in both clones, for ´CMC-40´ as auxin and for ´Señorita´ as cytokinin, makes think the endogenous auxin content in this latter was lower so an exogenous addition of this regulator was needed in the culture medium to equal the values of the control treatment indicators.

From these results, it necessary to emphasize that the use of Pectimorf® favored the *in vitro* establishment of cassava tips (*Manihot esculenta* C) in the clones ´CMC- 40´ and ´Señorita´, however, its effect was not identical in both genotypes which proves product's influence at the time of establishing any methodology involving changes of regulators and concentrations to the culture medium.

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BIBLIOGRAPHY

1. Ghanem, H. *The state of food insecurity in the world.* [en línea], (eds. Dawe, D., Stamoulis, K., y Wiebe, K.), edit. Electronic Publishing Policy and Support Branch Communication Division FAO, Rome, Italy, 2009, ISBN 978-92-5-106288-3, [Consultado: 8 de septiembre de 2015], Disponible en: <http://www.fao.org/docrep/012/i0876e/i0876e00.htm>.
2. Nassar, N.M.A.; Junior, O.P.; Sousa, M.V. y Ortiz, R. ''Improving Carotenoids and Amino-Acids in Cassava'', *Recent Patents on Food, Nutrition & Agriculture*, vol. 1, no. 1, 1 de enero de 2009, pp. 32-38, ISSN 2212-7984, 1876-1429.
3. Pérez, J.L. ''Proyección Estratégica hasta el 2015'', *Programa Integral de los Cultivos Varios*, 1.ª ed., edit. Liliana, La Habana, Cuba, 2010, p. 95, ISBN 978-959-7111-55-9.
4. Santana, M.A.; Romay, G.; Matehus, J.; Villardón, J.L. y Demey, J.R. ''Simple and low-cost strategy for micropropagation of cassava (*Manihot esculenta* Crantz)'', *African Journal of Biotechnology*, vol. 8, no. 16, 2009, ISSN 1684-5315, DOI 10.4314/ajb.v8i16.62061, [Consultado: 8 de septiembre de 2015], Disponible en: <http://www.ajol.info/index.php/ajb/article/view/62061>.
5. Medero, V.V.; Borroto, C.; Rodríguez, S.; Gómez, R.; López, J.; García, M.; Ventura, J.; Espinosa, J.; Cabrera, M.; Martínez, M.; Torres, M.; Torres, Y.; Álvarez, M. y García, J. ''Embriogénesis somática a partir de meristemos axilares en yuca'', *Biotecnología Vegetal*, no. 1, 2000, pp. 27-32, ISSN 2074-8647.
6. Onuoch, C.I. y Onwubiku, N.J.C. ''Micropropagation of cassava (*Manihot esculenta* Crantz) using different concentration of Benzyaminiopurin (BAP)'', *Journal of Engineering and Applied Sciences*, vol. 2, no. 7, 2007, pp. 1229-1231, ISSN 1816-949x, 1818-7803.
7. Li, H.-Q.; Sautter, C.; Potrykus, I. y Puonti-Kaerlas, J. "Genetic transformation of cassava (*Manihot esculenta* Crantz)", *Nature Biotechnology*, vol. 14, no. 6, junio de 1996, pp. 736-740, ISSN 1087-0156, DOI 10.1038/nbt0696-736.
8. Medina, R.D.; Faloci, M.M.; Neffa, V.S. y Mroginski, L.A. ''Embriogénesis somática y regeneración de plantas de mandioca (*Manihot esculenta* Crantz) de cultivares de interés para Argentina'', *Revista de Investigaciones Agropecuarias*, vol. 32, no. 3, 2003, pp. 143-160, ISSN 0325-8718, 1669-2314.
9. Ochoa, J.C.; Chavarriaga, P.; López, C. y others ''Embriogénesis somática y producción de callo embriogénico friable de dos cultivares de yuca (*Manihot esculenta* Crantz)", *Revista Colombiana de Biotecnología*, vol. 14, no. 2, 2012, pp. 20–27, ISSN 0123-3475.
10. Mapayi, E.F.; Ojo, D.K.; Oduwaye, O.A. y Porbeni, J.B.O. ''Optimization of In-Vitro Propagation of Cassava (*Manihot esculenta* Crantz) Genotypes'', *Journal of Agricultural Science*, vol. 5, no. 3, 2013, p. 261, ISSN 1916-9760, DOI 10.5539/jas.v5n3p261.
11. Cabrera, J.; Iglesias, R. y Hormaza, J. *Procedimiento de obtención de una mezcla de oligosacáridos pécticos estimuladora del enraizamiento vegetal*, Instituto Nacional de Ciencias Agrícolas, Cuba, no. 155, 2003.

12. Hernández-Mata, G.; Mellado-Rojas, M.E.; Richards-Lewis, A.; López-Bucio, J.; Beltrán-Peña, E. y Soriano-Bello, E.L. ''Plant Immunity Induced by Oligogalacturonides Alters Root Growth in a Process Involving Flavonoid Accumulation in Arabidopsis thaliana'', *Journal of Plant Growth Regulation*, vol. 29, no. 4, 30 de mayo de 2010, pp. 441-454, ISSN 0721-7595, 1435-8107, DOI 10.1007/s00344-010-9156-x.
13. Galletti, R.; Ferrari, S. y Lorenzo, G.D. ''Arabidopsis MPK3 and MPK6 Play Different Roles in Basal and Oligogalacturonide- or Flagellin-Induced Resistance against *Botrytis cinerea*'', *Plant Physiology*, vol. 157, no. 2, 1 de octubre de 2011, pp. 804-814, ISSN , 1532-2548, DOI 10.1104/pp.111.174003, [PMID: 21803860].
14. Hernández, M.M.; Suárez, L. y Valcárcel, M. ''Empleo del Pectimorf en la micropropagación de *Spathiphyllum* sp.'', *Cultivos Tropicales*, vol. 30, no. 3, septiembre de 2009, pp. 56-58, ISSN 0258-5936.
15. Hernández, A.; Ascanio, M.O.; Morales, M. y León, A. *La historia de la clasificación de los suelos de Cuba*, edit. Félix Varela, La Habana, Cuba, 2006, p. 98, ISBN 959-07-0145-0.
16. Hernández, R.M.; Diosdado, E.; Coll, F. y Cabrera, J.C. ''Efecto de los biorreguladores del crecimiento en la embriogénesis somática demandarina Cleopatra (*Citrus reshni* Hort. ex Tan.)'', *Cultivos Tropicales*, vol. 31, no. 3, septiembre de 2010, pp. 00-00, ISSN 0258-5936.
17. Hernández, A.; Pérez, J.; Bosch, D. y Castro, N. *Clasificación de los suelos de Cuba 2015*, edit. Ediciones INCA, Mayabeque, Cuba, 2015, p. 93, ISBN 978-959-7023-77-7.
18. Murashige, T. y Skoog, F. ''A revised medium for rapid growth and bio assays with tobacco tissue cultures'', *Physiologia Plantarum*, vol. 15, no. 3, 1962, pp. 473–497, ISSN 1399-3054.
19. *IBM SPSS Statistics* [en línea], versión 17, [Windows], edit. IBM Corporation, U.S, 2011, Disponible en: <http://www.ibm.com>.
20. Buchanan, B.B.; Gruissem, W. y Jones, R.L. *Biochemistry & Molecular Biology of Plants*, 1.ª ed., edit. American Society of Plant Physiologists Rockville, MD, Maryland, USA, 22 de marzo de 2002, p. 1408, ISBN 978-0-943088-39-6.
21. Pernisová, M.; Klíma, P.; Horák, J.; Válková, M.; Malbeck, J.; Souček, P.; Reichman, P.; Hoyerová, K.; Dubová, J.; Friml, J. ''Cytokinins modulate auxin-induced organogenesis in plants via regulation of the auxin efflux'', *Proceedings of the National Academy of Sciences*, vol. 106, no. 9, 2009, pp. 3609–3614, ISSN 0369-8211, 2250-1746.
22. Bari, R. y Jones, J.D.G. ''Role of plant hormones in plant defence responses'', *Plant Molecular Biology*, vol. 69, no. 4, 16 de diciembre de 2008, pp. 473-488, ISSN 0167-4412, 1573-5028, DOI 10.1007/s11103-008-9435-0.
23. Lalitha, N.; Kih, S.; Banerjee, R.; Chattopadhya, S.; Saha, A.K. y Bindroo, B.B. ''High frequency multiple shoot induction and in vitro regeneration of mulberry (*Morus indica* L. cv. S-1635)'', *International Journal of Advanced Research*, vol. 1, 2013, pp. 22–26, ISSN 2320-5407.
24. Suárez, L.; Cervone, F.; Hernández, M.M. y Sánchez, M. ''Aclimatización de plántulas de yuca (*Manihot esculenta*). Aporte al estudio de los mecanismos de acción de Pectimorf'', *IX Congreso Internacional de Biotecnología Vegetal*, Ciego de Ávila, Cuba, 2013, ISBN 978-959-16-2045-3.
25. Hernández, M.M.; Suárez, L.; Castilla, Y.; González, M.E.; Valcárcel, M. y López, M. ''Empleo de una mezcla de oligogalacturónidos en la micropropagación de la malanga (*Colocasia* sp.), var. «Camerum 14»'', *XV Congreso Científico del INCA*, edit. Ediciones INCA, Mayabeque, Cuba, 2006, ISBN 959-7023-36-9.
26. González, O.; Falcón, A.; Hernández, M.; Iglesias, R.; Silva, J.J.; López, M.; Rodríguez, J.E.; Arias, L.; Cabrera, J.C. y Oliva, E. ''Evaluación del efecto del Pectimorf® en el establecimiento in vitro de yemas axilares de boniato clon CEMSA 78-354'', *Biotecnología Vegetal*, vol. 4, no. 2, 2004, pp. 115–119, ISSN 1609-1841, 2074-8647.
27. Cid, M.; González-Olmedo, J.L.; Lezcano, Y. y Nieves, N. ''Influencia del Pectimorf sobre la calidad de la semilla artificial de la Caña de azúcar (*Saccharum* sp.)'', *Cultivos Tropicales*, vol. 27, no. 1, 2006, pp. 31–34, ISSN 0258-5936, 1819-4087.

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