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RESPONSE OF *Stevia rebaudiana* Bertoni TO THE APPLICATION OF BIOACTIVE PRODUCT PECTIMORF®

Respuesta de *Stevia rebaudiana* Bertoni a la aplicación del producto bioactivo Pectimorf®

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ABSTRACT. *Stevia rebaudiana* Bertoni is a shrub that has sweetening properties on its leaves and can be used for medicinal purposes. The quality and vigor of the cuttings are essential for the later transplanting and acclimatization of the crop in the nursery phase, reason why it is important to find alternatives that guarantee an efficient rooting. The objective of the work was to evaluate the effect of Pectimorf® on the growth of *Stevia* cuttings in the nursery stage. The cuttings were soaked for two different times (30 and 60 minutes) at 10 and 20 mg L^{-1} concentrations of Pectimorf®. As a control the conventional hormone indoleacetic acid (IAA) (30 minutes at 1 mg L^{-1}) and water were used as absolute control. Evaluations were performed from 20 to 50 days in the nursery. The addition of Pectimorf® to the concentration of 20 mg L^{-1} increased the roots and leaves number, stem diameter, dry mass of leaves and stems, as well as Brix degrees in leaves of the apical zone on plants. The effect of Pectimorf® imbibition time at this concentration depended on the evaluated variable.

Key words: rooting, cuttings, bioproducts, nursery

RESUMEN. *Stevia rebaudiana* Bertoni es una planta arbustiva que posee propiedades edulcorantes en sus hojas y que puede ser utilizada con fines medicinales. La calidad y el vigor de los esquejes resultan esenciales para el posterior trasplante y aclimatación del cultivo en la fase de vivero, por lo que es importante encontrar alternativas que garanticen un eficiente enraizamiento. El objetivo del trabajo fue evaluar el efecto del Pectimorf® en el crecimiento de esquejes de *Stevia* en la fase de vivero. Los esquejes se embebieron durante dos tiempos diferentes (30 y 60 minutos) a las concentraciones de 10 y 20 mg L^{-1} de Pectimorf®. Como control se emplearon la hormona convencional ácido indolacético (AIA) (30 minutos a 1 mg L^{-1}) y el agua como testigo absoluto. Se realizaron evaluaciones desde los 20 y hasta los 50 días en semillero. La adición de Pectimorf® a la concentración de 20 mg L^{-1}, incrementó el número de raíces y hojas, el diámetro del tallo, la masa seca de hojas y tallos, así como los grados Brix en hojas de la zona apical de las plantas. El efecto del tiempo de imbibición del Pectimorf® a esta concentración dependió de la variable evaluada.

Palabras clave: enraizamiento, esquejes, bioproductos, vivero

INTRODUCTION

The fresh leaves of *Stevia* possess stevioside and rebaudioside, both are glycosides that attribute the sweet taste that can be up to 300 times more than sucrose, it is used as a substitute for synthetic sweeteners, in addition, they do not provide calories. Therefore it can be used in the food and pharmaceutical industry of several countries to sweeten various products as a substitute for synthetic sweeteners. This plant has other beneficial functions in health, helps regulate blood pressure, eliminate toxins, is antimicrobial and antioxidant and has digestive, diuretic and antacid properties (1).

Therefore, it is necessary from the phytotechnical point of view to deepen the study and to perfect the technology of this species in the current conditions of Cuba; however, few studies are from the agronomic point of view, hence the novelty in the different lines of research to be developed (2).

Considering that *Stevia* is an asexual reproduction plant, the need arises to produce a high number of cuttings to achieve its multiplication and to have plants to meet the current demands of companies and producers interested in its cultivation.

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To achieve this, products of a chemical nature of high cost worldwide are generally used, such as indoleacetic acid (AIA); therefore, it is necessary to find alternatives that guarantee an efficient rooting and a positive effect on the growth and development of the plants.

In this sense, among the products of plant origin with attributes for agriculture, is Pectimorf®, a product obtained from a mixture of oligogalacturonides. The works consulted refer to their ability to influence different physiological processes that stimulate the growth and development of plants (3). According to this background, the objective of this work was to evaluate the Pectimorf® product as a stimulator of the rooting and growth of Stevia plants in the nursery phase.

MATERIALS AND METHODS

The research was developed in experimental areas of the National Institute of Agricultural Sciences (INCA), located in the municipality of San José de las Lajas, Mayabeque province, Cuba, in a period that covers from July 14, 2014 to January 9, 2015.

The substrate used was a mixture of organic fertilizer (cow dung) and rice husk, in a 3: 1 ratio (4). Table 1 shows the chemical characteristics of the substrate that was used. The pH and interchangeable cations are in the right range for the development of the crop (5).

Analyzes were carried out in the laboratory of Biofertilizers and Nutrition of the INCA plants, according to the methodology described (6). The trial was set up with Stevia plants, free of pests. Considering the uniformity of the cuttings, propagules of 10 cm long with five pairs of leaves were used. The cuttings were planted in trays of 70 alveoli with a diameter of 5.5 cm and a depth of 4.5 cm. The Pectimorf® obtained by the Bioactive Products Group of the Department of Plant Physiology and Biochemistry of INCA was used, from the enzymatic degradation of pectin from the bark of citrus fruits.

This has as its active ingredient a mixture of oligogalacturonides of pectic origin with a degree of polymerization between 9 and 16 (7), which provides among its additional advantages, solubility in the aqueous medium.

The cuttings were imbibed in two concentrations and two times of imbibition. The concentrations used were 10 mg L^{-1} and 20 mg L^{-1} and the imbibition time was 30 and 60 minutes. Distilled water and AIA (indoleacetic acid) at the concentration of 1 mg L^{-1} were used as control for 30 minutes, giving rise to six treatments (Table 2), under a completely randomized experimental design, constituting 15 plants for each repetition treatment in study.

Table 2. Description of the treatments (T) tested

Treatments-imbibition
Control (water)
Control AIA 1 mg L^{-1} 30 min
Pectimorf® 10 mgL^{-1} 30 min
Pectimorf® 10 mg L^{-1} 60 min
Pectimorf® 20 mg L^{-1} 30 min
Pectimorf® 20 mg L^{-1} 60 min

To the plants of each treatment, at 20 and 40 days after the imbibition (DDI), the following evaluations were made:

Number of roots (U): visual count of the roots present in each of the treatments. It was only determined at 20 DDI.

Radical length (cm): the main root was measured with a graduated rule.

Number of leaves per plantlet (U): by visual count, the height of the seedlings (cm), was measured with a graduated rule, from the neck of the root to the axilla of the youngest leaf.

Diameter of the stem of the seedlings (mm): was determined with a vernier caliper, from two centimeters above the neck of the root.

Dry mass of the seedlings (g): drying in an oven (BrBOXUN) at a temperature of 700 °C for seven days until constant mass. It was weighed in analytical balance (Sartorius).

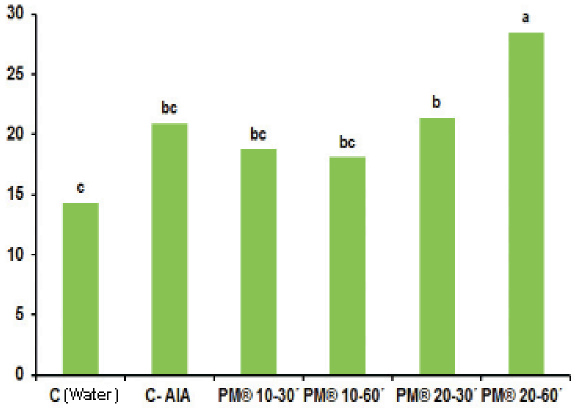
The data obtained were analyzed by means of a simple classification ANOVA. The resulting means were compared with the Duncan Multiple Range Test for $p \leq 0.05$ when there were significant differences between the treatments. The Statgraphics Centurion-2013 program was used under the Windows 7 operating system.

Table 1. Chemical characteristics of the substrate of the trays

pH (H_2O)	MO (%)	Exchangeable cations					
		Ca (cmol kg^{-1})	Mg (cmol kg^{-1})	P (ppm)	K (cmol kg^{-1})	Na (cmol kg^{-1})	∑
7,0	42,5	26,5	23	1951	4,6	2,82	76,4

RESULTS AND DISCUSSION

One of the most important variables in the planting of cuttings is the number of roots that are able to form, since it will depend in part, the anchoring in the soil at the time of transplantation. In Figure 1 it can be seen that 20 days after the imbibition of the cuttings (moment of the transplant), those that were imbibed in 20 mg L^{-1} of Pectimorf®, during 60 minutes, had a greater number of roots, value that differed statistically from the other treatments and, above all, the one that included the AIA, plant growth stimulating hormone. The lowest number of roots was obtained with the control treatment, which evidences the need to use a product that stimulates the growth of plants.



Means with the same letters do not differ significantly according to Duncan p <0.05 *

DAI- days after imbibition

Figure 1. Roots by *S. rebaudiana* cuttings at 20 DAI

In accordance with this result, also the potentialities of Pectimorf® as in raiser, has shown ability to induce the formation of adventitious roots in African violet petioles and carnation cuttings, obtaining a remarkable effect on root formation, with values up to 12 units, after 20 days of imbibition and, therefore, greater success in the development of the plants (8). As for the growth regulators (synthetic compounds, their plant hormones), these condition the development of the physiological processes in the cutting and stimulate radical initiation (4).

The practical application of these products has as an objective the stimulation of root emission, which guarantees the homogeneity of rootedness; accelerate rooting and increase the percentage of survival.

On the other hand, in the reproduction by cuttings, the induction of rooting comprises complex anatomical and physiological processes, in which an important role plays the combined action of the auxins and the rooting cofactors that are synthesized in the leaves and buds (9).

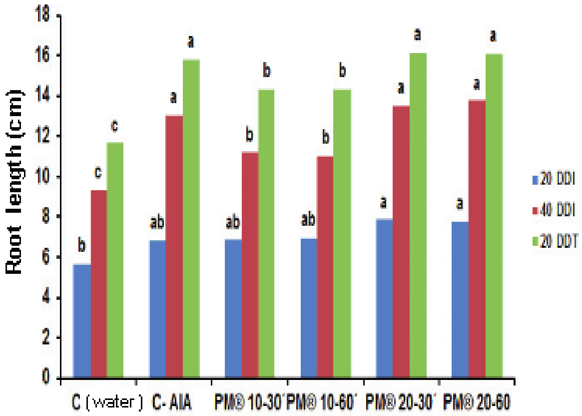
Finally, the phases of initiation and radical branching are recognized anatomically by the development of callus in the cuttings and the emission of lateral roots in developed primordia (10). Both stages have a marked influence on the propagation by cuttings (11). From the productive point of view they mark the initial and final time for carrying out the transplant to the stock market or to the definitive site. The fact that the cuttings without application of product, that is, cuttings embedded in water, also emit roots, suggests the expression of their intrinsic capacity for this physiological activity; already recognized capacity, by noting that in the cuttings there are also centers producing auxins, such as leaf apices and stems and axillary buds.

Auxin in these centers can be transported as free auxin, without physiological activity, through the conduits of the cutting, to act in the places of greatest demand (12).

Figure 2 shows the length of the roots evaluated at different times of plant growth. The length of the roots was greater with the application of the AIA and the Pectimorf® compared with the imbibition of cuttings in water. The Pectimorf®, at the concentration of 10 mg L^{-1} in the 2nd and 3rd did not reach values similar to those obtained by the application of the traditional hormone (AIA); and with the application of 20 mg L^{-1} no significant differences were observed in relation to the AIA (Figure 2).

These results are in agreement with studies carried out in the cultivation of guava with the dose of 20 mg L^{-1} of Pectimorf®, which shows that this product can totally replace the traditional growth regulators like the AIA and in many of the cases, superior results are obtained (4).

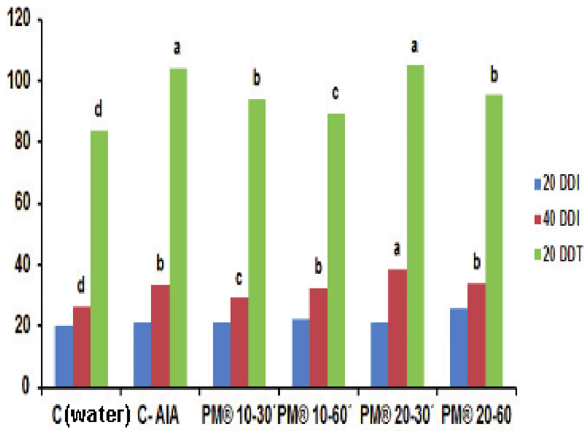
The leaf of this crop is the organ with the highest content of sweetener, so it is important to have a large number of them. In addition, they have great importance in the regulation of the capacity and photosynthetic efficiency of the plant and are optimizers of the distribution of assimilates that constitute fundamental indicators for the increase of the yields.



DDI- days after imbibition
DDT- days after transplant
Means with equal letters do not differ significantly according to Duncan <0.05*

Figure 2. Length of the radical system of *S. rebaudiana* cuttings at 20 and 40 DAI and 20 DAT

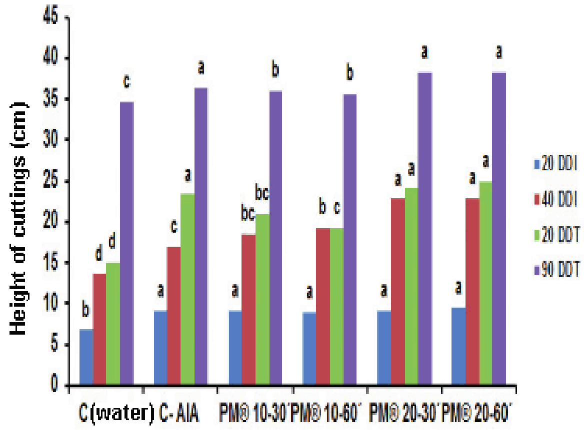
In Figure 3 it can be seen that the number of leaves increases substantially, from the first to the third evaluation, as is characteristic of these plants in this phase. In the last two evaluations, statistical differences between the different treatments are shown. The highest number of leaves, in the second evaluated moment, was obtained with the application of 20 mg L^{-1} of Pectimorf® during 30 minutes, with higher values and different from the rest of the treatments; while the lower concentration of the product did not induce an increase of this variable in the magnitude obtained with the traditional hormone, but it was superior to the control.



Means with equal letters do not differ significantly according to Duncan <0.05*
DDI- days after imbibition
DDT- days after transplant

Figure 3. Number of cuttings leaves of *S. rebaudiana* at 20 and 40 DDI and 20 DDT

The height of the plants can be seen in Figure 4, in which the treatments were different from each other. All treatments induced an increase in height in relation to water control. In the second evaluation, the cuttings that were imbibed during 60 minutes in both concentrations of Pectimorf® as well as those that were imbibed during 30 minutes in 20 mg L^{-1}, showed higher values than those obtained by the AIA; however, in the third and fourth evaluation moments, the application of the highest concentration of Pectimorf® stimulated the height at the same level as the AIA.

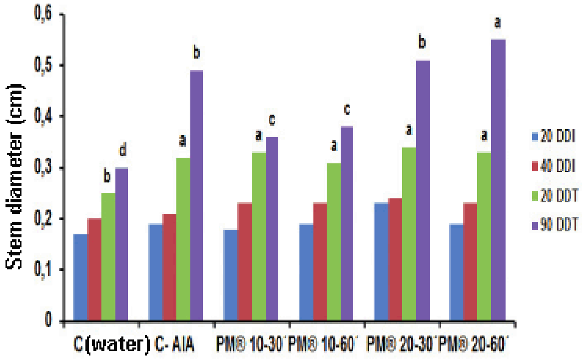


Means with equal letters do not differ significantly according to Duncan <0.05*
DDI- days after imbibition
DDT- days after transplant

Figure 4. Height of cuttings of *S. Rebaudiana* at 20 and 40 DAI, 20 and 90 DAT

Work done with Oligogalacturonides (OGs) refers that a possible way by which the height of the plant is increased is because the oligosaccharins can stimulate the photosynthetic activity; therefore, there is a greater gain of carbon skeletons that can be used for the synthesis of new compounds, such as proteins (13).

Stem diameter is one of the key parameters in the choice of postures and cuttings to produce plants in optimum condition (14). In particular, the application of GOs has been shown to favor this variable in different crops (15). Figure 5 shows the results of the application of Pectimorf® on the diameter of the cuttings, with significant differences only in the last two evaluations, where the applications of AIA and Pectimorf® showed increases in relation to the control.



Means with equal letters do not differ significantly according to Duncan p<0.05*
DDI- days after imbibition, DDT- days after transplantation

Figure 5. Diameter of the stem of cuttings of *S. rebaudiana* at 20 and 40 DAI, 20 and 90 DAT

Similar results reported that when applying 20 mg L^{-1} of PectiMorf® to guava cuttings (*Psidium guajava* L.), an increase of this variable was observed when it was combined with different supports such as rice straw and zeolite (4). In general, Pectimorf® has been shown to function as a hormonal chemical messenger that regulates the mechanisms of growth and differentiation in different crops (16), so that the increase in stem diameter observed can be one of the variables on which positively influence One of the most important variables to evaluate in crops is the dry mass of the different organs of the plant, due to the high correlation that it has with the growth of the same. In Table 3 the effect of Pectimorf® on the dry mass of the stem of the plants is observed.

Table 3. Dry mass of the cuttings stem of *S. rebaudiana* at 20 and 40 DDI, 20 and 90 DDT

Treatments	20 DDI	40 DDI	20 DDT	90 DDT
	grams (g)			
Control (water)	0,03	0,07 c	0,25 c	3,40 c
Control (AIA)	0,02	0,10 b	0,37 ab	5,46 a
Pectimorf® 10 mg L^{-1} (30 min)	0,03	0,11 ab	0,32 b	4,13 b
Pectimorf® 10 mg L^{-1} (60 min)	0,03	0,13 ab	0,36 ab	4,21 b
Pectimorf® 20 mg L^{-1} (30 min)	0,03	0,13 ab	0,33 ab	5,13 a
Pectimorf® 20 mg L^{-1} (60 min)	0,03	0,15 a	0,40 a	5,53 a
ESx	0,003	0,008*	0,02*	0,39*

Means with equal letters do not differ significantly according to Duncan p<0.05*
DDI- days after imbibition, DDT- days after transplantation

In the first evaluation the treatments were not different from each other, contrary to what happened in the second, mainly at the end of the first vegetative cycle (40 DAI), where the dose of 20 mg L^{-1} with 60 minutes of imbibition of Pectimorf® , achieved an increase of the dry mass by 100% in relation to the control. With this result it can be inferred that the plants that received the application of the products, make a better use of the nutrients and water that results in the greater formation of biomass in the same period of time, compared to those that do not receive this treatment.

Other results showed that foliar spraying of areca palm plants with 2.10 and 20 mg L^{-1} caused its maximum plant response point in the 10 mg L^{-1} dose (17).

While, when using the product for the rooting of two varieties of guava, supreme Red and Cuban Red Dwarf, obtained the best vegetable responses with 10 and 20 mg L^{-1} respectively (4). Table 4 shows the accumulation of dry matter in the leaves, which had a behavior similar to that of the stem, with the difference that in this case, the Pectimorf® 20 mg L^{-1} treatment during 30 minutes was statistically different from the treatment with auxin, exceeding it by 39%. This result allows to affirm that this oligogalacturonide is biologically active at the concentration studied, which characterizes this group of biomolecules in a new hormonal hierarchy.

Table 4. Dry mass of cuttings leaves of *S. rebaudiana* at 20 and 40 DAI, 20 and 90 DAT

Treatments	20 DDI	40 DDI	20 DDT	90 DDT
	grams (g)			
Control (water)	0,23	0,30 c	0,86 b	6,23 c
Control (AIA)	0,19	0,33 b	1,05 a	8,93 b
Pectimorf® 10 mg L^{-1} (30 min)	0,22	0,41 ab	0,93 ab	9,20 b
Pectimorf® 10 mg L^{-1} (60 min)	0,24	0,39 ab	1,03 ab	10,06 ab
Pectimorf® 20 mg L^{-1} (30 min)	0,21	0,43 a	0,94 ab	12,46 a
Pectimmorf® 20 mg L^{-1} (60 min)	0,25	0,44 a	1,05 a	10,06 ab
ESx	0,01 ns	0,02*	0,05*	0,39*

Means with equal letters do not differ significantly according to Duncan p<0.05*
DAI- days after imbibition, DAT- days after transplantation

The greater formation of dry mass obtained in some cases with the Pectimorf® could translate into a higher photosynthetic efficiency of the plants that would lead to yield increases. The values of the dry mass of the root give measure of its growth and its development when applying the different products. The results presented in Table 5 show a similar tendency to the behavior of the variables related to dry mass, previously evaluated.

Table 5. Dry mass of the roots of *S. rebaudiana* cuttings at 20 and 40 DAI, 20 and 90 DAT

Treatments	20 DDI	40 DDI	20 DDT	90 DDT
		grams (g)		
Control (water)	0,06	0.20 c	0,33 c	5,26 c
Control (AIA)	0,05	0,29 ab	0,62 a	7,66 a
Pectimorf® 10 mg L^{-1} (30 min)	0,05	0,26 ab	0,43 b	7,00 b
Pectimorf® 10 mg L^{-1} (60 min)	0,05	0,29 ab	0,40 b	6,93 b
Pectimorf® 20 mg L^{-1} (30 min)	0,05	0,30 ab	0.59 a	7,73 a
Pectimorf® 20 mg L^{-1} (60 min)	0,06	0,32 a	0,41 b	7,86 a
ESx	0,007* NS	0,02*	0,03*	0,64*

Means with equal letters do not differ significantly according to Duncan p<0.05*
DAI- days after imbibition, DDT- days after transplant

The dry mass of the roots showed significant differences at the time of evaluation, from the 40 DAIs, when using Pectimorf® and AIA treatments.

CONCLUSIONS

- The largest rooting of Stevia cuttings is achieved with the 20 mg L^{-1} dose of Pectimorf® through the imbibition of the cuttings, for 30 and 60 minutes, which increased the growth of the plants during the phase of nursery.
- The product was an economically viable alternative as a substitute for conventional hormones, such as the AIA.

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