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EFECT OF PECTIMORF® ON ROOTING AND *In Vitro* ACCLIMATIZATION OF PAPAYA (*Carica papaya* L.) SHOOTS CULTIVAR MARADOL ROJA

Efecto del Pectimorf® en el enraizamiento y la aclimatización *in vitro* de brotes de papaya (*Carica papaya* L.) cultivar Maradol Roja

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ABSTRACT. *In vitro* propagation of papaya has been successfully reported by different researchers, however the problems in the process of rooting and acclimatization have not been completely resolved yet. The low percentage of rooting and low survival of plants under conditions of acclimatization remain a problem for the development of an efficient micropropagation protocol for this crop. Employment growth regulator cuban Pectimorf® could be used to minimize this problem. In two experiments that were conducted as treatments are made up of two concentrations of sucrose (0 and 10 g L^{-1}), two of auxin AIB (0 and 2 mg L^{-1}) and five Pectimorf® concentrations (3, 5, 7, 9 and 12 mg L^{-1}). Were used as controls: the culture medium rooting composed of MS salts 50 %, 2 mg L^{-1} IBA, 0,4 mg L^{-1} thiamine, sucrose 40 g L^{-1} agar 7 g L^{-1} and the same culture medium without sucrose and Pectimorf®, but with zeolite as carrier. The results showed that the Pectimorf® had a positive effect on rooting and *in vitro* acclimatization of papaya shoot. The synergistic action of AIB with 9,0 mg L^{-1} Pectimorf® allowed to obtain *in vitro* plants with greater leaf area, fresh weight, number of roots, photosynthetic rate and stomatal conductance, which together with a high percentage of rooting and less percentage of open stomata allowed to reach a 76,2 % survival *ex vitro* conditions.

Key words: auxin, oligosaccharides, papaya, roots, zeolite

RESUMEN. La propagación *in vitro* de la papaya ha sido exitosamente informada por diferentes investigadores; sin embargo, los problemas en los procesos de enraizamiento y aclimatización aún no han sido completamente resueltos. Los bajos porcentajes de enraizamiento y la baja supervivencia de las plantas en condiciones de aclimatización continúan siendo un problema para el desarrollo de un eficiente protocolo de propagación *in vitro* en este cultivo. El empleo del regulador del crecimiento cubano Pectimorf® podría ser utilizado para minimizar este problema. En el trabajo se realizaron dos experimentos que tuvieron como tratamientos dos concentraciones de sacarosa (0 y 10 g L^{-1}), dos de la auxina AIB (0 y 2 mg L^{-1}) y cinco concentraciones de Pectimorf® (3, 5, 7, 9 y 12 mg L^{-1}). Se emplearon como controles: el medio de cultivo de enraizamiento compuesto por las sales MS al 50 %; 2 mg L^{-1} de AIB; 0,4 mg L^{-1} de tiamina; 40 g L^{-1} de sacarosa; 7 g L^{-1} de agar y el mismo medio de cultivo sin sacarosa, sin Pectimorf® pero con zeolita como soporte. Los resultados demostraron que el Pectimorf® tuvo un efecto positivo en el enraizamiento y la aclimatización *in vitro* de los brotes de papaya. La acción sinérgica del AIB con 9 mg L^{-1} de Pectimorf® permitió obtener plantas *in vitro* con mayor área foliar, masa fresca, número de raíces, tasa fotosintética y conductancia estomática; lo cual unido a un alto porcentaje de enraizamiento y un menor porcentaje de estomas abiertos permitió alcanzar un 76,2 % de supervivencia en condiciones *ex vitro*.

Palabras clave: auxina, oligosacáridos, papaya, raíces, zeolita

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INTRODUCTION

Papaya (*Carica papaya* L.) is a native crop of Central America that is characterized as short-term and continuously productive throughout the year, from the economic point of view. Its high yields and nutritional

value rank among tropical fruit jewels with important feeding and medicinal applications (1).

Red Maradol is still the most important cultivar in Cuba, grown in a total area of 5,396 ha with a production of 105 562 t in 2015[A].

Although papaya plants regenerated via somatic embryogenesis have been successful, the biggest worldwide problem is *in vitro* culture acclimation of regenerated plants (2).

In vitro propagation processes are implemented in a large physical extension for growing plants, mainly on shelves with closed flasks containing nutritive culture media under aseptical conditions, so as to avoid fungal or opportunist bacterial contamination. These conditions make *in vitro* grown plants very sensitive to rough environmental changes, for instance, when they are transferred to *ex vitro* conditions under natural environments (3).

As a result of *in vitro* environment, plants have a different anatomy and physiology from those grown under field conditions or greenhouses (4-6), because of their organ disorders, although not all of them have the same effect on *ex vitro* behavior. Among these disorders are the poor development of leaf cuticle photosynthetic apparatus, the non-functional emerged roots disconnected from conductive bundles and others that may affect plant survival during acclimation phase (7).

The term acclimation is defined as the environmental adaptation of plants obtained by tissue culture or *in vitro* propagation that have been transferred to a new environment, greenhouses or the field. During acclimation, the environment is gradually changed to plants with time, beginning by the closest *in vitro* environment and ending by the nearest greenhouse or field environment. *Ex vitro* acclimation is performed in greenhouses or the field under shade conditions, whereas *in vitro* acclimation can be completed in culture flasks during photo-autotrophic propagation (8).

Several studies have been conducted so far focused on autotrophic systems with forced ventilation, so that plants are better prepared to come out. The increased light intensity in flasks, CO_2 concentration and forced ventilation are aspects that help encourage autotrophic nutrition, always removing sucrose, which allows reducing production costs at the conventional *in vitro* propagation and microbial contamination in the culture medium (8-10). Commonly, high CO_2 concentrations increase photosynthesis and vegetative growth under *ex vitro* conditions (11).

However, previous studies conducted on *in vitro* papaya propagation have revealed that the main difficulties to propagate this species are rooting and plant acclimation in greenhouses (12, 13). Plant survival may reach values between 65 and 70 % under these conditions during the first seven days of *ex vitro* culture. The most important thing is that plants are capable of building a good root system, because nutrition largely depends on their root performance. Therefore, new biotechnological strategies should be settled to increase the efficiency of *in vitro* propagation protocols of papaya plantlets, cv. Red Maradol.

The introduction of active substances from national production in the methodology of *in vitro* plant regeneration could be an alternative to improve *in vitro* rooting. Pectimorf® can be mentioned among these active substances, which is a mixture of (1-4) α-D-oligogalacturonides with a polymerization degree between 9 and 16 (14).

Pectimorf® is recognized as a new Cuban bioregulator derived from citrus industry wastes, whose active ingredient is a mixture of oligosaccharides of peptic origin. Pectimorf® ability to induce and develop rooting as well as significantly increase *in vitro* plant development and vigor in different crops is worthy as a promising choice in plant biotechnology (14-16). Consequently, the objective of this research work was to determine the effect of a Cuban product called Pectimorf® on *in vitro* plant rooting and acclimation of papaya, a bioregulator that has not been yet used for such purpose in this species.

MATERIALS AND METHODS

Research studies were conducted at the Institute of Plant Biotechnology from "Marta Abreu" Central University of Las Villas, Santa Clara, Cuba.

In vitro papaya (*Carica papaya* L. cv. Red Maradol) shoots obtained from somatic embryos were used as planting material. They had four subcultures in the extension culture medium (17) containing these salts: MS at 100 % of its concentration; 1,0 mg L^{-1} thiamine; 1,2 uM 6-benzyl amine purine (6 BAP);

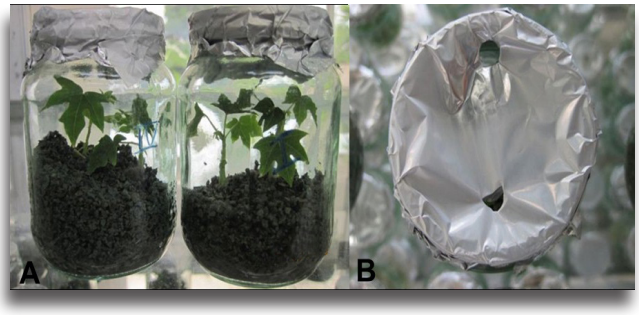
[A] MINAG (2016) Estadísticas oficiales del Ministerio de la Agricultura. La Habana, Cuba. Cierre diciembre 2015.

1,5 uM naphthalene acetic acid (NAA); 100 mg L^{-1} myo-inositol; 30 g L^{-1} sucrose; 0,06 mg L^{-1} vitamin B_2 and 5 g L^{-1} Agar gel (Sigma Co) (18). Shoots of 3,0-4,0 cm long were selected for the trials, removing its basal leaves and leaving just the youngest four leaves.

Culture conditions

Glass culture flasks of 250 mL capacity with transparent plastic caps were used at the control treatment with agar in both experiments. Flasks containing liquid culture medium and zeolite were covered with a sheet of aluminum foil, besides adding 30 mL culture medium to them. Each treatment had 33 replications, planting two shoots per culture flask that were grown inside heated rooms at a temperature of 27±2 °C with sunlight, a photoperiod of 13/11 h light/darkness and a photosynthetic photon flow ranging between 48,0 and 62,5 umol m^{-2} s^{-1}, measured by means of an Extech 401025 (Extech Instruments, USA) light meter. Experiments were repeated twice.

After three days of culture, a hole was opened in the sheet of aluminum foil covering culture flasks of different treatments and experiments, in order to increase ventilation with the help of sterile forceps. A second hole was opened after four days (Figure 1).



(A) culture flask with shoots at the beginning of experiments (B) holes made in the sheet of aluminum foil covering flasks to increase ventilation

Figure 1. *In vitro* papaya (*Carica papaya* L. cv. Red Maradol) shoots in culture medium with zeolite as substrate

Effect of combining sucrose, IBA and Pectimorf® on *in vitro* rooting and acclimation

The goal of this first experiment was to determine the effect of combining sucrose with or without indole butyric acid (IBA) growth regulator and various Pectimorf® concentrations for *in vitro* shoot rooting. Sterile zeolite was used as substrate, a natural aluminum silicate with excellent ion exchange properties with a high absorption and granulation power of 3/1 mm (Table I). Then, 97 g of this mineral were added to each glass culture flask and covered with a sheet of aluminum foil (Figure 1).

Table I. Physic-chemical characteristics of natural zeolite. Tasajera deposit, Villa Clara

Chemical composition	(%)
Silicon oxide (SiO_2)	70,10
Aluminum oxide III (Al_2O_3)	11,20
Iron oxide III (Fe_2O_3)	2,20
Iron oxide II (FeO)	0,30
Magnesium oxide (MgO)	0,60
Calcium oxide (CaO)	4,50
Sodium oxide (Na_2O)	1,50
Potassium oxide (K_2O)	1,30
Diphosphorus pentoxide (P_2O_5)	0,07
Water (H_2O)	4,70
Mineral composition	**(%)**
Clinoptilolite	40,00
Modernite	40,00
Others (calcite, quartz, feldspar)	20,00
Physical properties	Valor
Particle size	1,0-3,0 mm
Density (δ)	0,37 g cm^{-3}
Density of solid phase (γ)	1,77 g cm^{-3}
Total porosity (PT)	80,59 % vol

Three Pectimorf® concentrations (3, 5 and 7 mg L^{-1}) were studied with and without sucrose (0 and 10 g L^{-1}) and IBA (2 mg L^{-1}), employing the following control treatments: a previously proposed rooting culture medium (17), containing MS salts at 50 % of its concentration; 2 mg L^{-1} IBA; 0,4 mg L^{-1} thiamine; 40 g L^{-1} sucrose; 7 g L^{-1} agar, pH 5,8 prior to autoclaving and the same culture medium but with zeolite; 2 mg L^{-1} IBA and without sucrose.

Effect of combining IBA-Pectimorf® without sucrose on *in vitro* rooting and acclimation

The second objective of this experiment was to evaluate higher Pectimorf® concentrations combined with IBA, taking into account the above experimental results and using the following Pectimorf® concentrations: 7, 9 and 12 mg L^{-1}.

the control treatment in this experiment was the previously described rooting culture medium supplemented just with 2 mg L^{-1} IBA and zeolite as substrate.

In this experiment, a group of 20 plants per treatment were transferred to *ex vitro* conditions during the acclimation phase to evaluate their survival.

For evaluating plant morphological and physiological indicators, 20 shoots were randomly selected per treatment at 37 days of its culture. Moreover, contamination percent was evaluated in 100 % culture flasks with both holes and controls.

Morphological and physiological variable evaluations

Upon ending *in vitro* experiments, the following morphological evaluations were performed to shoots and plants: plant height (cm); leaf number; internode number; rooting (%); root number; root length (cm); *in vitro* fresh weight (g FW); presence or absence of basal callus.

In addition, for the second experiment, stomatal number per mm^2 and percentage of open and closed stomata were evaluated at 12 noon in samples taken from 10 *in vitro* plants (37 days of culture) and 10 *ex vitro* plants (seven days during acclimation phase), leaf area (by the proposed method for papaya plants (19), along with some physiological indicators, such as net photosynthetic activity, total transpiration and stomatal conductance.

Stomatal observation

Stomata were observed on abaxial leaf surface through Engleman's modified replication method (20), which consists of putting a drop of Kola Loka® superglue (Kola Loka SA, Mexico) on a slide, then pressing the leaf on the drop for one minute and soon taking it off the slide. The prints were carried to the laboratory where they were observed under an Anjue N-800 (Shanghai, China) optical microscope that had one HDCE-50B (Alltion, China) digital camera adapted. Three fields chosen at random per treatment were photographed for counting stomata.

Determination of photosynthesis, total transpiration and stomatal conductance

Fully expanded leaves (leaves no. 2 and 3) at the same position in shoots and *in vitro* plants were selected at the end of the experiment, about four to five hours after photoperiod onset. Determinations were made per treatment in 12 plants wit 10 measurements each, for a total of 120 measurements. Maximum photosynthetic capacity (umol CO_2 m^{-2} s^{-1}), total transpiration (mmol H_2O m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1}) were measured by CIRAS-2 equipment (Portable Photosynthesis system, UK) coupled to a PLC6 2,5 cm^2 universal tray, whose area was completely covered with the leaf (1,7 cm^2). Carbon dioxide concentration, air temperature and relative humidity (80-90 %) were environmental values. To work with such equipment, light was set at an intensity of 900 umol m^{-2} s^{-1}. Measurements were always made to all *in vitro* plants from 9:00 to 10:00 a.m.

Furthermore, water use efficiency of photosynthesis was calculated by the fórmula: CO_2 net uptake/stomatal conductance (21).

Ex vitro acclimation conditions

Plants were grown under a 12h-light/12h-darkness photoperiod, with an average daytime temperature of 30±2 °C and a relative humidity of 70-75 %. Light intensity ranged between 224 and 457 umol m^{-2} s^{-1} that was measured with an Extech 401025 light meter (Extech Instruments, USA).

A substrate containing zeolite (Table I) and sugarcane (*Saccharum* spp hybrid) filter cake compost (9:1, v/v) was used. These components were placed in plastic pots of 500 mL total volume. Compost was first deposited on the bottom and zeolite over it to ensure good aeration to plant roots of papaya. Irrigation was manually done by spraying twice daily. Plants were covered with a clear glass flask for five days to assure a greater relative humidity than 90 and 70 % shading with a black saran mesh.

Then, 20 plants were taken per treatment, combining IBA with 9 and 12 mg L^{-1} Pectimorf®, whereas controls in zeolite and agar without Pectimorf®. Survival rate (%) was determined by counting living plants at the time of evaluation (seven days) (22).

Statistical analysis

SPSS program package for Windows version 21 was used for statistical analysis (23); Shapiro-Wilk test to analyze normality of variables, the nonparametric alternative of variance analysis, Kruskal-Wallis, to compare means and the nonparametric Mann-Whitney test to compare between pairs of groups.

RESULTS AND DISCUSSION

Effect of combining sucrose, IBA and Pectimorf® on *in vitro* rooting and acclimation

Control treatment with zeolite (without sucrose and 2 mg L^{-1} IBA) had a greater response to rooting percentage than the other treatments. Regarding other morphological variables evaluated, it had the same or higher values with significant differences in various treatments. None of Pectimorf® concentrations used in this first experiment (3, 5 and 7 mg L^{-1}) exceeded control treatment results; however, it is worth noting that all of them surpassed the control with agar and 40 g L^{-1} sucrose (Table II).

For those variables related to rooting, such as root number and rooting percentage, the values reached by control treatment with zeolite and 2 mg L^{-1} IBA were higher than the rest with significant differences.

Pectimorf® concentrations combined or not with IBA auxin in this experiment did not promote root length or number. Just 50 % rooting was recorded by the treatment without sucrose, 2 mg L^{-1} IBA and 7 mg L^{-1} Pectimorf® with a lower value than 62,5 % control treatment with zeolite and 2 mg L^{-1} IBA (Table II).

In treatments without sucrose in the culture medium, plants usually grew and developed as in treatments with sucrose (10 g L^{-1} and control 40 g L^{-1}). This implies that *in vitro* papaya plants had a photoautotrophic behavior under these conditions (Table II). However, it is important to note that when there is sucrose in the culture medium, despite both growth stimulators, rooting percentage was very low (5,0 %) or null.

Sucrose removed from the culture medium not always presupposes an adequate root system development in all *in vitro* grown plants.

It is reported that *Doritaenopsis* orchid plants growing in culture medium with sucrose showed a

Table II. Effect of combining sucrose, IBA and Pectimorf® on *in vitro* plant growth and rooting of papaya (*Carica papaya* L. cv. Red Maradol) growing in culture flasks with zeolite as substrate at 37 days of culture

Sucrose (g L^{-1})	IBA (mg L^{-1})	Pectimorf® concentration (mg L^{-1})	Height (cm)	Leaf number	Fresh weight (gMF)	Internode number	Root length (cm)	Root number	Rooting (%)
0	0	3	3,58 b	3,78 cd	0,42 bc	10,14 a	0,12 b	0,28 c	5,00 d
0	0	5	4,19 a	4,55 b	0,47 b	11,05 a	0,01 c	0,05 d	5,00 d
0	0	7	3,88 ab	3,90 bc	0,50 ab	10,20 a	0,18 ab	0,10 d	5,00 d
0	2	3	4,13 a	3,69 cd	0,60 a	12,70 a	0,11 b	0,54 c	28,6 c
0	2	5	3,68 b	4,18 b	0,52 ab	11,87 a	0 d	0 c	0 e
0	2	7	4,02 a	4,18 b	0,52 ab	11,87 a	0,32 a	1,81 b	50,0 b
10	2	3	4,00 a	3,44 d	0,46 b	8,88 b	0 d	0 c	0 e
10	2	5	3,38 c	4,00 b	0,33 c	8,00 b	0 d	0 c	0 e
10	2	7	3,36 c	3,66 cd	0,32 c	7,11 b	0,04 c	0,11 d	5,00 d
0 Control Zeolite	2	0	4,24 a	6,03 a	0,73 a	9,68 ab	0,58 a	2,03 a	62,5 a
40 Control Agar	2	0	3,62 b	3,26 d	0,42 bc	9,26 ab	0 d	0 c	0 e
Significance			*	*	*	*	*	*	*

Means with uncommon letters within the same column differ statistically according to Kruskal-Wallis/Mann-Whitney nonparametric test for P<0,05 (n= 20)

greater number of longer roots than the treatment without sucrose (24).

The use of IBA as a growth regulator of root formation is known on *in vitro* papaya culture (13, 17, 25); however, this regulator in a semisolid culture medium stimulates basal callus formation that prevents or makes some roots nonfunctional, having no connection to the stem. No basal callus was formed in shoots from any treatment with zeolite as substrate, despite of IBA at a concentration of 2 mg L^{-1} combined with Pectimorf®. Regarding *in vitro* papaya culture, Pectimorf® has only been studied at low concentrations (1 mg L^{-1}) combined with 6 BAP at *in vitro* propagation (organogenesis) within the establishment and multiplication phases[B].

Zeolite also allowed better root growth and development, due to a greater aeration during *in vitro* growth. These results had not been previously obtained with this crop. Nevertheless, vermiculite has been used as substrate for shoot rooting at *in vitro* papaya culture.

The use of vermiculite combined with IBA for rooting different varieties of papaya has been reported by several authors (25, 26), who indicate its beneficial effect to reach high rooting percentages (80-90 %). However, some authors point out its higher effect on other auxins (ANA; IAA) to achieve *in vitro* papaya shoot rooting, but in a semisolid culture medium (13).

On the other hand, microbial contamination levels reached 10,0-15,0 % in culture flasks with sucrose in the medium at 37 days of culture, whereas its values were lower than 4,0 % in the other treatments without sucrose.

Results from the first experiment led to study higher Pectimorf® concentrations to increase rooting percentage of *in vitro* papaya cv. Red Maradol shoots together with IBA without sucrose in the culture medium and using zeolite as substrate.

Effect of combining IBA-Pectimorf® without sucrose on *in vitro* rooting and acclimation

Treatments with the highest Pectimorf® concentrations of 9 and 12 mg L^{-1} (Figure 2) showed a better response to a morphological variable (leaf area), with significant differences compared to the rest of treatments. Concerning the longest root variable, the highest values were recorded in both aforementioned treatments without statistical differences. However, the greatest percentage of rooted plants (84,2 %) was achieved at Pectimorf® concentration of 9 mg L^{-1} complemented with IBA auxin, it being superior to other treatments and the control at 37 days of culture (Table III).

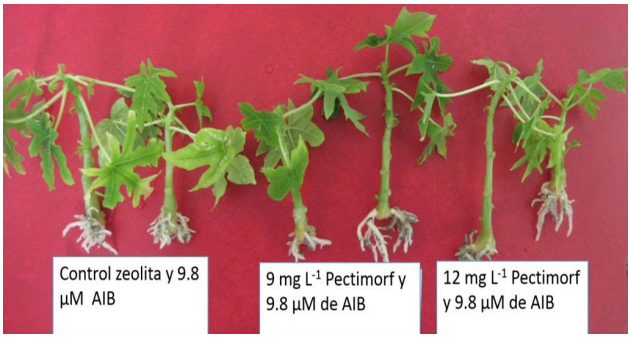


Figure 2. Appearance of *in vitro* papaya cv. Red Maradol plants and its root system in the control treatment and the highest Pectimorf® concentrations studied at 37 days of culture in flasks with zeolite as substrate and two holes on the cap to increase ventilation

This study proved that the best Pectimorf® concentration promoting papaya shoot root formation is the same as its concentrations used in other crops. For instance, in cassava (*Manihot esculenta* L.)[C], the best results on *in vitro* shoot rooting and acclimation were obtained with 9 mg L^{-1} Pectimorf®. It also promoted *in vitro* shoot root development in *Spathiphyllium* sp (15) and secondary roots in violet (*Saintpaulia ionantha*) petioles, that is, an auxin effect of using oligogalacturonic mixture at 10 mg L^{-1} (16) compared to indole acetic acid auxin (IAA), which did not increase root length or number, as *in vitro* papaya shoots.

Other authors state that Pectimorf® has the same action as auxins or a synergy with auxin in the culture medium (27). These experimental results showed Pectimorf® synergy with IBA auxin.

[B] Roque, L. A. Y. *Propagación in vitro de la papaya* (*Carica papaya* L.) *cv Maradol Roja: Una alternativa de solución para los productores habaneros*. [Tesis de Doctorado], Universidad Agraria de La Habana, Facultad de Agronomía, La Habana, Cuba, 2004, 100 p.

[C] Hernández, H. M.; Suarez, L.; Valcárcel, M. y López, M. ''Empleo de una mezcla de oligogalacturónidos (Pectimorf) en la micropropagación de yuca (*Manihot esculenta*, Crantz) y Malanga (*Colocasia* sp.)''. En: *II Simposio Internacional de Raíces, Rizomas, Tubérculos, Plátanos, Bananos y Papaya*, edit. Instituto Nacional de Investigaciones en Viandas Tropicales (INIVIT), Villa Clara, Cuba, 2013.

Table III. Effect of combining IBA-Pectimorf® on *in vitro* plant growth and rooting of papaya (*Carica papaya* L. cv. Red Maradol) growing in culture flasks with zeolite as substrate at 37 days of culture

Sucrose (g L^{-1})	IBA (mg L^{-1})	Pectimorf® concentration (mg L^{-1})	Height (cm)	Leaf number	Leaf area (cm^2)	Fresh weight (gMF)	Internode number	Root length (cm)	Root number	Rooting (%)
0	2	7	4,02	4,57	1,32 b	0,56 b	12,77 a	0,35	2,07	52,8 c
0	2	9	4,04	5,21	1,59 a	0,80 a	10,21 ab	0,75	2,15	84,2 a
0	2	12	4,06	5,21	1,50 a	0,84 a	10,72 ab	0,87	1,72	69,0 b
0	2	0	4,27	6,09	1,30 b	0,69 ab	9,61 b	0,55	2,00	63,6 b
Control Zeolite										
Significance			n.s	n.s	*	*	*	n.s	n.s	*

Means with uncommon letters within the same column differ statistically according to Kruskal-Wallis/Mann-Whitney nonparametric test for P<0,05 (n= 20)

The longest root was reached in tobacco (*Nicotiana tabacum* L.) and *Arabidopsis thaliana* (L.) Heynh (28) by using 10 mg L^{-1} Pectimorf® combined with IBA at a lower concentration (0,5 mg L^{-1}) than in this work. These authors report that Pectimorf® stimulated taproot elongation and slowed down lateral root formation. Its concentrations combined with IBA did not promote a larger root number per shoot of *in vitro* papaya culture (Tables II and III).

When analyzing the results of all physiological variables evaluated (photosynthesis, transpiration and stomatal conductance), Pectimorf® treatments had a negative effect on *in vitro* papaya photosynthetic activity at 37 days of culture.

The control treatment (zeolite and 2 mg L^{-1} IBA) reached the highest value of photosynthesis and stomatal conductance with significant differences compared to the others; however, it had a greater transpiration, which is a negative effect on *in vitro* plant acclimation to *ex vitro* conditions, since they will have to undergo a bigger water loss (Table IV).

When analyzing water use efficiency in photosynthesis, the highest value was achieved by Pectimorf® concentration of 12 mg L^{-1}, compared to other treatments, so proving that papaya plants were taking up more CO_2 with less water (Table IV).

The treatment with 9 mg L^{-1} Pectimorf® had lower levels of photosynthesis and stomatal conductance compared to zeolite control, but higher than the rest of its concentrations with significant differences, in addition to less transpiration than the control and a more effective response under *ex vitro* conditions with good values of crop survival (Table V).

With regard to open and closed stomata relationship as well as its total number per mm, the highest percentages of open stomata were recorded at *in vitro* plants of control treatments (zeolite and agar as substrate) with 35,0 % and 72,41 %, respectively. However, the values were lower in treatments with Pectimorf® and IBA auxin, which is a positive aspect for the subsequent adaptation to *ex vitro* conditions.

This enables to confirm that papaya plants have been subjected to a process of *in vitro* acclimation with Pectimorf®. Besides, stomatal number per area was lower than other treatments at 9 mg L^{-1} Pectimorf® (Table V). What is above mentioned along with a higher percentage of rooted plants in the treatment with 9 mg L^{-1} allowed to obtain the highest survival rate (76,2 %) in seven days under *ex vitro* acclimation conditions, despite that the treatment with 12 mg L^{-1} showed greater water use efficiency in photosynthesis, which demonstrates the importance of getting a great number of *in vitro* rooted plants to ensure high survival rates under *ex vitro* conditions.

It is important to point out plant response at the control treatment with agar, because they did not have roots as a result of *in vitro* culture conditions with high relative humidity inside culture flasks; plants had a high percentage of open stomata (72,4 %) and although they did not have a high stomatal number per area, 100 % of them could not survive under *ex vitro* acclimation conditions (Table V).

Authors reported that when comparing leaf stomatal density of *in vitro* grown plants with those in greenhouses found more than double stomatal density on *in vitro* plants that in greenhouses (29).

They also found differences in stomatal size and number. *In vitro* plants from a woody species (*Castanea sativa*) had a higher amount

Table IV. Pectimorf® effect on photosynthetic activity (umol CO_2 m^{-2} s^{-1}), transpiration (mmol H_2O m^{-2} s^{-1}), water use efficiency in photosynthesis and stomatal conductance (mmol m^{-2} s^{-1}) of *in vitro* plants of papaya (*Carica papaya* L. cv. Red Maradol) grown in flasks with zeolite as substrate

Sucrose (g L^{-1})	IBA (mg L^{-1})	Pectimorf® concentration (mg L^{-1})	Photosynthesis (µmol CO_2 $m^{-2}s^{-1}$)	Transpiration (mmol H_2O $m^{-2}s^{-1}$)	Water use efficiency (µmol CO_2 mmol H_2O^{-1})	Stomatal conductance (mmol m^{-2} s^{-1})
0	2	7	1,481 d	0,666 d	2,223	12,14 c
0	2	9	3,548 b	1,298 b	2,733	24,26 b
0	2	12	3,070 c	0,793 c	3,871	18,66 b
0 Control Zeolite	2	0	3,828 a	1,506 a	2,542	34,85 a
Significance			*	*		*

Means with uncommon letters within the same column differ statistically according to Kruskal-Wallis/Mann-Whitney nonparametric test for P<0,05 (n= 20)
Measurements were made to plants remaining for 37 days under *in vitro* acclimation conditions

Table V.Leaf stomata and survival of *in vitro* papaya (*Carica papaya L.* cv. Maradol Roja) plants at 37 days of culture and seven days after planting under acclimatization conditions

Sucrose (g L^{-1})	IBA (mg L^{-1})	Pectimorf® concentration (mg L^{-1})	Open stomata (%)		Closed stomata (%)		Stomatal number (mm^2)		Survival (%)
			0 días	7 días	0 días	7 días	0 días	7 días	
0	2	9	26,14	66,60	73,86	33,40	87	69	76,2
0	2	12	33,30	71,76	66,67	28,40	117	60	50,8
0 Control Zeolite	2	0	35,00	24,50	65,00	73,60	100	60	58,9
40 Control Agar	2	0	72,41	0,0	27,59	0,0	88	0	0,0
		Seeded plants		27,5		72,5		40	100,0

of open stomata than those in greenhouses. Such amount was changed in this research, due to the conditions that allowed *in vitro* plant acclimation as photoautotrophism (culture medium without sucrose), zeolite as substrate, holes on flask caps for a better aeration and high *ex vitro* plant survival.

Pectimorf® has been used by various authors for rooting in different plant species grown *in vitro* culture (15, 16, 30), who point out that the adequate Pectimorf® concentration as growth regulator for *in vitro* culture is around 10 mg L^{-1}, which supports the results of this work.

This oligogalacturonide had a synergy action together with auxin in the culture medium, which was proved by *in vitro* shoot rooting of papaya reaching 20,6 % more rooted shoots with the best combination of IBA and Pectimorf® (9 mg L^{-1}) compared to the control without Pectimorf®. One part of this positive effect on growth is attributed to high cell division rates. However, Pectimorf® mechanisms that promote cell division in higher plants are still unknown (28).

The synergistic action of IBA with 9 mg L^{-1} Pectimorf®, coupled with the growing conditions used, enabled to obtain *in vitro* papaya plants with greater leaf area, fresh weight and root number, high photosynthetic rate and stomatal conductance, which together with a higher rooting percentage (84,2 %) and a smaller percentage of open stomata than other treatments allowed to reach 76,2 % plant survival under *ex vitro* conditions.

CONCLUSIONS

- Pectimorf® had a positive influence on *ex vitro* papaya plant rooting and acclimation.
- The largest number of rooted plants as well as the highest percentage of plant survival was achieved by the treatment with 9 mg L^{-1} Pectimorf® combined with 2 mg L^{-1} IBA without sucrose in the culture medium and using zeolite as substrate.

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