academicJournals

Vol. 14(35), pp. 2622-2635, 2 September, 2015
DOI: 10.5897/AJB2015.14814
Article Number: 21DC9EB55388
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Effects of different culture conditions (photoautotrophic, photomixotrophic) and the auxin indole-butyric acid on the *in vitro* acclimatization of papaya (*Carica papaya* L. var. Red Maradol) plants using zeolite as support

Laisyn Posada Pérez[1], Yenny Padrón Montesinos[1], Justo González Olmedo[2], Romelio Rodríguez Sánchez[2], Osvaldo Norman Montenegro[3], Raul Barbón Rodriguez[1], Ortelio Hurtado Ribalta[1] Rene Carlos Rodriguez Escriba[2], Dion Daniels[4] and Rafael Gómez-Kosky[1]*

[1]Instituto de Biotecnología de las Plantas. Universidad Central "Marta Abreu" de Las Villas. Carretera a Camajuaní Km 5 ½, Santa Clara, Villa Clara. Cuba.
[2]Centro de Bioplantas. Universidad de Ciego de Ávila, Carretera a Morón km 9, Ciego de Ávila, Cuba.
[3]Centro de Bioactivos Químicos. Universidad Central "Marta Abreu" de Las Villas, Carretera a Camajuaní Km 5 ½. Santa Clara, Villa Clara, Cuba.
[4]University of Belize, Hummingbird Avenue, Belmopan, Cayo District, Belize, Central America.

Received 23 June, 2015; Accepted 31 August, 2015

Plant regeneration of papaya via organogenesis and somatic embryogenesis has been successful; however, the biggest problem of *in vitro* culture of this species is the acclimatization of regenerated plants, where over 70% of the plants are lost before being planted in the field. Decreasing the relative humidity inside the culture vessel and thus increasing the ventilation, appears to have a greater effect on the adaptation of papaya plants, strengthening the function of the stomata and with this, allowing better control of water loss from the leaves. The aim of this study was to determine the effects of different concentrations of sucrose and indole-butyric acid (IBA) on rooting and *in vitro* acclimatization of plants using sterile zeolite as support and culture vessels with increased ventilation. Three concentrations of sucrose (0, 10 and 20 g L^{-1}) were studied with and without auxin and as the control treatment, the rooting culture medium with agar during 17, 27 and 37 culture days. The highest percentage of rooting was recorded at 37 culture days in the treatment without sucrose and IBA with 80.0% and zeolite as support. The best photosynthetic values were achieved when *in vitro* shoots were grown in culture medium with auxin and different concentrations of sucrose, even though they were also high in the treatment without the presence of IBA and without sucrose at 17 days of culture. The combined effect of the zeolite, auxin (IBA), without sucrose in the culture medium and increased ventilation allowed photoautotrophic culture conditions which had effect of the increasing plant survival under *ex vitro* acclimatization conditions.

Key words: *Carica papaya*, photosynthesis, roots formation.

INTRODUCTION

In Cuba, the most commercially important variety is the 'Red Maradol' and crop production exceeded 1.7 million tons in 2013 (FAOSTAT, 2014). In addition, it is sown in other countries in the Caribbean and Central America. Regeneration of papaya plants via somatic embryogenesis has been successful; however, the somatic embryos in germination have problems with root development due to the presence of a basal callus, which prevents the formation of roots or its connection to the stem, besides the low percentage of acclimatization of rooted plants (Fitch and Manchardt, 1990; Dhekney et al., 2007; Sekeli et al., 2013). Another critical aspect has been the adaptation to environmental conditions because of the high relative humidity that this species need to achieve high survival rates (Chen et al., 1991). The biggest problem that exists globally in *in vitro* papaya culture is the *ex vitro* acclimatization of regenerated plants, where more than 70% of *in vitro* plants produced are lost before being planted in the field (Malabadi et al., 2011). A plant that originated *in vitro*, differs in many aspects from those formed *in vivo* (Pierik, 1990), since its environmental conditions, substrate, light, and nutrition, are very different. It is also important to note that the growth *in vitro* is heterotrophic, while the conditions *in vivo* are autotrophic. The *in vitro* atmosphere, with a high relative humidity, low or zero gas exchange, shortage of CO_2 during most of the period, ethylene production and low photosynthetic rate, induce changes in plants grown under these conditions. After transferring the plants to *ex vitro* environment, plants have to correct all of these abnormalities in order to acclimatize to the new environment, either in greenhouse or into the field (Kadleček et al., 2001).

Furthermore, the anatomy of the leaves is influenced by light and humidity, differing anatomically from those originated from *in vivo* conditions (Brainerd et al., 1981). Because of this, the acclimatization is an important factor in the subsequent survival rates of the plants, since it is a critical stage in the process, in which the larger loss occurs. In this stage, the relative humidity should begin to decrease gradually, to allow in addition to stomata closure, better cuticle formation and reduced water loss. Moreover, for best results in *in vivo* establishment, it is necessary to have root *in vitro* development (Pierik, 1990). Decreasing the relative humidity inside the culture vessel and thus the increased ventilation, appear to have a greater effect on the adaptation of grape plants (*Vitis vinifera* L.), enhancing the stomata function and thereby enable better control of water loss from the leaves (Gribaudo et al., 2001).

In vitro photoautotrophic can be induced excluding carbohydrates from the culture medium and increasing gas exchange in the culture vessel (Kozai, 2010; Xiao et al., 2011). Photoautotrophic micropropagation is defined as micropropagation without sucrose in the culture medium, where the accumulation of carbohydrates in *in vitro* tissues cultured and their subsequent growth is completely dependent on photosynthesis and inorganic nutrients (Zobayed et al., 2004; Kozai, 2010). Therefore this may also be called photosynthetic micropropagation in culture medium devoid of sugar (Xiao et al., 2011). In photoautotrophic micropropagation, acclimatization can also be completed in the culture vessel, which is called *in vitro* acclimatization (Kozai et al., 2005).

Although the growth and physiological changes in some plant species with photoautotrophic growth have been studied (Norikane et al., 2010; Badr et al., 2011; Shin et al., 2013), there are very few reports of studies on the *in vitro* propagation of papaya and none specifically on its most critical phase - rooting. For this reason, this study aims to evaluate the effects of different concentrations of sucrose and the auxin indole-butyric acid to achieve *in vitro* acclimatization in a growth chamber with sunlight, greater ventilation of the culture vessels and using zeolite as a support for increased survival rates *ex vitro* of papaya plants obtained by somatic embryogenesis.

MATERIALS AND METHODS

Plant material and culture media

As plant material, *in vitro* shoots of papaya - variety Maradol Roja were used. These were regenerated from somatic embryos, originating from the fourth subculture in the elongation culture medium proposed by Posada-Perez et al. (2007). This culture medium contained Murashige and Skoog (MS) (1962) salt at 100% concentration supplemented with 1 mg L^{-1} of thiamine, 1.2 µM of 6-benzyl aminopurine (BAP), 1.5 µM of naphthaleneacetic acid (NAA), 100 mg L^{-1} of myo-inositol, 30 g L^{-1} of sucrose, 1 µM of riboflavin and 5 g L^{-1} of Agargel (Sigma Co.) and adjusted to a pH of 5.8. Shoots with a size between 3.0 to 5.0 cm in length, of which only the last three leaves were left, were placed in culture vessels containing three concentrations of sucrose (0, 10 and 20 g L^{-1}) combined with the presence or absence of the growth regulator, indole-butyric acid (IBA) at a concentration of 9.8 µM for *in vitro* rooting and acclimatization of shoots, using as support to the mineral zeolite (natural aluminum-silicate with excellent ionic exchange properties and a high absorption power) 1 to 3 mm granulation (Table 1). To each glass culture vessel, 97 g of zeolite

*Corresponding author. E-mail: kosky@ibp.co.cu, kosky2015@gmail.com.

Abbreviations: IBA, Indole-butyric acid; **BAP,** 6-benzyl aminopurine; **NAA,** naphthaleneacetic acid.

Table 1. Physical-chemical characteristics of natural zeolite (Tasajera Deposit, Villa Clara, Cuba).

Chemical composition	**(%)**
Silicon oxide (SiO_2)	70.10
Aluminium 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
Mordenite	40.00
Others (Calcite, quartz, feldspar)	20.00
Physical properties	**Value**
Size of the particle	1.0-3.0 mm
Density (δ)	0.37 g cm^{-3}
Density of the solid phase (γ)	1.77 g cm^{-3}
Total porosity (TP)	80.59% vol.

previously sterilized in an oven at 180°C for 2 h were added. Glass culture vessels with a total volume of 250 mL with 30 mL of liquid culture medium were used. They were covered with aluminum foil of 20 µm thickness. After three days of culture, the ventilation of the culture vessels was increased by opening holes on the aluminum foil covering the culture vessels in the different treatments. A second hole was made three days after the opening of the first (Figure 1A). As the control treatment, a modified version of the culture medium for rooting proposed by Posada-Perez et al. (2007) was used. This culture medium was composed of MS salts at 50% concentration, 9.8 µM of IBA, 0.4 mg L^{-1} of thiamine, 1.0 µM of riboflavin, 40 g L^{-1} of sucrose, 7.0 g L^{-1} of agar and pH of 5.8 prior to sterilization. The culture vessels and the volume of culture medium were the same as previously mentioned, but these were covered with plastic lid (polycarbonate). Forty five vessels were used with two shoots each per each variant of culture medium. Of each variant 15 culture vessels were randomly selected every 10 days, from 17 to 37 days of culture for evaluations of the morphological and physiological indicators of plants, including contamination. Survival (%) in *ex vitro* acclimatization conditions was done with plants after 17 days of *in vitro* culture conditions. This evaluation was done 20 days after being transplanted.

Culture conditions

The culture vessels with the shoots were placed in growth rooms at a temperature of 27 ± 2°C with sunlight and a photoperiod of 13 / 11 h, light / dark with a light intensity ranging between 48.0 and 62.5 µmol m^{-2} s^{-1}; measured with a light meter EXTECH 401,025 (USA). The experiments were repeated twice. The relative humidity inside the culture vessel covered with aluminum foil and two holes was between 72 to 68% and in control with culture vessel lids with plastic was 90 to 85%.

Evaluation of morphological and physiological variables

After 17, 27 and 37 days of culture, *in vitro* shoots and plants were evaluated for the following morphological variables: length of the plant (cm), number of leaves, number of internodes, fresh mass of *in vitro* plant (gFM), leaf area (by the method proposed by Cardona et al. (2009) to estimate the leaf area of papaya plants), presence or absence of basal callus, number of roots, length of the roots (cm) and presence of roots (%). For the evaluations of the physiological indicators of *in vitro* shoots and plants, the contents of the pigments chlorophyll a, b and carotenoids were determined at 17, 27 and 37 days of culture using the same plants which had been used to assess the morphological variables. To determine the net photosynthetic activity and total transpiration, *in vitro* plants at 17 days of culture were used.

Chlorophyll and carotenoid pigments measurement

At 27 days, chlorophyll a, chlorophyll b and total carotenoid pigments were determined in the leaves from *in vitro* plants using the Meyer-Berthenrath´s method, modified by Stirban (1985). The absorbance of the extracts was measured at 663, 645 and 472 nm by spectrophotometry (GENESYS 6; Thermo Electronic Corporation Visionlite Vision 2.1),

Photosynthetic activity, total transpiration and stomatal conductance

For these measurements, fully extended leaves of the same position (leaves 2 and 3) of *in vitro* plants at the end of the experiment, 4 to 5 h after the start of the photoperiod were used. All measurements were performed with three leaves from different plants. The maximum photosynthetic capacity (µmol CO_2 m^{-2} s^{-1}), the total transpiration (mmol H_2O m^{-2} s^{-1}) and stomatal conductance (mmol m^{-2} s^{-1}) were measured with the equipment CIRAS-2 (Portable Photosynthesis System, UK), coupled to a universal bucket PLC6 2.5 cm^2. The area of the tray was completely covered with the leaf (1.7 cm^2). The concentration of CO_2, air temperature and relative humidity (80 to 90%) were environmental values taken into consideration. The light equipment, intensity was set at 900 µmol m^{-2} s^{-1}. Measurements were always done on all *in vitro* plants between 9:00 to 10:00 a.m.

Ex vitro acclimatization conditions

The environmental acclimatization conditions are characterized by averaged daytime temperature of 30 ± 2°C, 65 to 70% relative humidity and light intensity ranging between 224 and 457 µmol m^{-2} s^{-1} measured with a light meter EXTECH 401 025. Plastic trays with cover were used and 100% zeolite as substrate with 15 *in vitro* plants per treatment, which had been cultured for the first 17 days (Figure 1B, C). These were kept closed during the first first days after transplanting to ensure high relative humidity above 90% and thereafter it was slowly reduced by opening the lid. Irrigation was done manually with aspersion three times daily. The trays were placed in a culture house with an internal black *Saran* mesh cover with 70% shade. The survival rate (%) was determined by counting the plants that were alive at the time of evaluation after 15 days with respect to the total number of plants initially planted.

Statistical analysis

For the statistical analysis of the data, the package SPSS version 17.0 for Windows 2008 was used. For analysis of the normality of

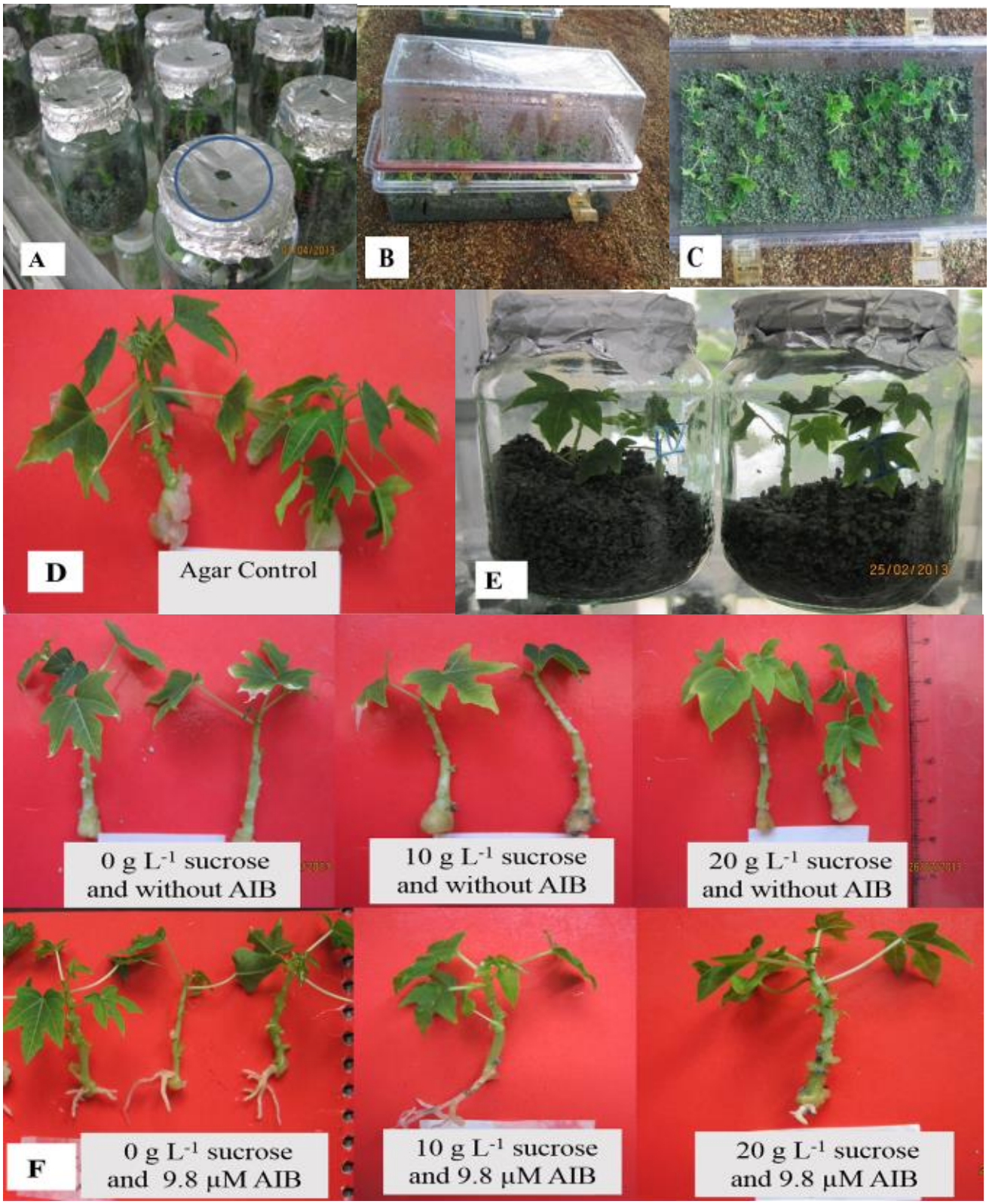

Figure 1. Rooting and *in vitro* acclimatization of papaya (*Carica papaya* L. var. Red Maradol) shoots obtained by somatic embryogenesis under different culture conditions. **(A)** Culture vessel with increased ventilation. **(B-C)** Plastic trays (polycarbonate) with zeolite as support used for *ex vitro* acclimatization plants after 17 days of *in vitro* culture. **(D)** Aspects of *in vitro* plants at the end of the experiment (37 days) in photoautotrophic culture conditions. **(E)** *In vitro* papaya plants with the formation of basal callus cultured in culture medium with agar and sucrose. **(F)** Stimulus of rhizogenesis in the presence or absence of auxin and sucrose at 37 days of culture.

Table 2. Effects of the interaction sucrose and IBA on growth and rooting of *in vitro* papaya (*Carica papaya* L. var. Red Maradol) plants growing in culture vessel with increased ventilation and zeolite as a support at 17 days of culture.

IBA (µM)	Sucrose (g L^{-1})	Height (cm)	No. of leaves	Leaf area (cm^2)	Fresh weight plant (gFW)	No. Internodes	Length of the roots (cm)	No. of roots	Rooting (%)	Survival (%)
0	0	3.63± 0.58^{a}	3.10±0.99^{c}	1.04±0.15^{a}	0.37±0.12^{b}	6.2±1.03^{a}	0.0^{b}	0.0^{c}	0.0^{c}	13.0^{c}
0	10	3.31±0.37^{a}	3.63±0.92^{b}	1.02±0.11^{a}	0.36±0.16^{b}	7.8±1.45^{a}	0.0^{b}	0.0^{c}	0.0^{c}	6.6^{c}
0	20	3.43±0.50^{a}	4.12±0.83^{ab}	1.00±0.14^{a}	0.40±0.26^{b}	8.5±2.44^{a}	0.0^{b}	0.0^{c}	0.0^{c}	0.0^{d}
9.8	0	3.20±0.27^{a}	3.70±0.82^{b}	1.08±0.12^{a}	0.20±0.12^{c}	7.9±1.73^{a}	0.14±0.18^{a}	0.50±0.53^{a}	40.0^{a}	60.0^{a}
9.8	10	3.26±0.33^{a}	4.25±1.16^{a}	1.05±0.14^{a}	0.29±0.99^{c}	9.3±2.55^{a}	0.13±0.35^{a}	0.13±0.35^{b}	13.3^{b}	33.0^{b}
9.8	20	3.86±0.43^{a}	3.00±0.53^{c}	1.00±0.15^{a}	0.48±0.28^{b}	11.4±4.92^{a}	0.0^{b}	0.0^{c}	0.0^{c}	20.0^{b}
9.8 (Agar control)	40	3.75±1.06^{a}	4.50±0.70^{a}	0.95±0.13^{a}	0.85±0.77^{a}	10.0±5.65^{a}	0.0^{b}	0.0^{c}	0.0^{c}	0.0^{d}

Different letters within a column indicate significant differences at $p \leq 0.05$ by Kruskal-Wallis/ Mann-Whitney test (n = 15). Values ± SD.

the variables, Shapiro Wilk test was used. For the comparison between means, the nonparametric alternative analysis of variances of Kruskal Wallis test was applied. For the comparison between pairs of groups, the nonparametric Mann Whitney test was used. A bi-factorial experimental design with four repetitions was performed, analyzing the data using a multi-factorial ANOVA. Differences between groups were detected by the test of least significant difference or LSD intervals. For the statistical analysis, the statistical program Stat-graphics Centurion 2007 version 15 software was used.

RESULTS AND DISCUSSION

Effects of sucrose and auxin on rooting and *in vitro* acclimatization

Results on management leading to increased rooting and which favor photoautotrophic culture conditions are shown in Tables 2, 3 and 4, which reflect the response of the different treatments on morphological variables recorded with increased ventilation during 10, 20 and 30 days. From the earliest morphological evaluations, the positive effect of the combination that represented the treatment without sucrose and 9.8 µM IBA over other treatments was significant. For the three times that *in vitro* papaya plants were measured, the largest number of roots and increased root length were obtained with the treatment with 10 g L^{-1} of sucrose and 9.8 µM of IBA, at 17 and 27 days of culture. It is also noteworthy that in the evaluation at 27 days of culture, high values in fresh mass were achieved in the *in vitro* plants grown in zeolite without sucrose and 9.8 µM IBA, although there were no significant differences observed in the treatments without sucrose and without IBA; with 10 g L^{-1} sucrose and without AIB and the agar control. This was due to, in the case of agar control, the presence of big basal callus (Figure 1D). The callus did not form or their presence was minimal in the other treatments evaluated (photoautotrophic and photomixotrophic conditions) using zeolite as a support and with increased ventilation in culture vessels regardless of the presence or absence of auxin in the *in vitro* culture medium. In this regard Kozai et al. (2005) reported that the species Calla Lily (*Zantedeschia elliottian* L.) in photoautotrophic culture conditions prevented the formation of basal callus of *in vitro* shoots, which is the cause of the poor rooting and limited uptake of water and nutrients by the plants. Zhang et al. (2009) in the Chinese medicinal species (*Momordica grosvenori* Swingle) indicated that no callus was formed in those plants grown in culture medium without sucrose, free of growth regulators and photoautotrophic culture conditions. With respect to the leaf area at 27 and 37 days of culture (Figure 1E) in the treatment without sucrose (photoautotrophic culture conditions) and with the presence of IBA, the *in vitro* plants showed higher values with significant differences with the other treatments (Tables 3 and 4).

Teixeira de Silva (2014) shows that photoautotrophic culture of *in vitro* plants was possible in papaya in two varieties (Rainbow and Sunrise Solo) using plants from *in vitro* germinated seed and transferred to culture vessels Vitron® type and using as support rock wool. To the culture vessels, constant CO_2 at a concentration of 3,000 ppm was added. In the photoautotrophic conditions evaluated, plants of both varieties had a higher number of leaves and number of roots with respect to the photohete-rotrophic and photomixotrophic treatments.

Table 3. Effects of the interaction sucrose and IBA on growth and rooting of *in vitro* papaya (*Carica papaya* L. var. Red Maradol) plants growing in culture vessel with increased ventilation and zeolite as a support at 27 days of culture.

IBA (µM)	Sucrose (g L^{-1})	Height (cm)	No. of leaves	Leaf area (cm^2)	Fresh weight plant (gFW)	No. Internodes	Length of the Roots (cm)	No. of roots	Rooting (%)
0	0	4.00±0.24^{a}	4.40±0.96^{a}	1.23±0.17^{b}	0.36±0.18^{a}	10.0±3.05^{a}	0.00^{b}	0.00^{b}	0.0^{c}
0	10	3.44±0.38^{a}	3.88±1.80^{b}	1.10±0.09^{c}	0.34±0.24^{a}	9.63±2.87^{a}	0.00^{b}	0.00^{b}	0.0^{c}
0	20	3.30±0.26^{a}	2.63±1.50^{c}	1.05±0.12^{d}	0.21±0.99^{b}	8.25±1.66^{a}	0.00^{b}	0.00^{b}	0.0^{c}
9.8	0	3.64±0.39^{a}	4.60±1.17^{a}	1.53±0.11^{a}	0.35±1.50^{a}	10.50±2.27^{a}	0.31±0.41^{a}	1.40±1.95^{a}	53.0^{a}
9.8	10	3.54±0.38^{a}	4.00±1.63^{b}	1.20±0.10^{b}	0.24±0.10^{b}	9.90±1.91^{a}	0.20±0.63^{a}	0.10±0.32^{b}	26.6^{b}
9.8	20	3.36±0.39^{a}	2.63±1.59^{c}	1.00±0.08^{d}	0.18±0.12^{c}	8.75±2.12^{a}	0.00^{b}	0.00^{b}	0^{c}
9.8 (Agar control)	40	3.55±0.21^{a}	4.50±0.70^{a}	1.00±0.09^{d}	0.30±0.70^{a}	9.50±1.41^{a}	0.00^{b}	0.00^{b}	0^{c}

Different letters within a column indicate significant differences at $p \leq 0.05$ by Kruskal-Wallis/ Mann-Whitney test (n = 15). Values ± SD.

Table 4. Effects of the interaction sucrose and IBA on growth and rooting of *in vitro* papaya (*Carica papaya* L. var. Red Maradol) plants growing in culture vessel with increased ventilation and zeolite as a support at 37 days of culture.

IBA (µM)	Sucrose (g L^{-1})	Height (cm)	No. of leaves	Leaf area (cm^2)	Fresh weight plant (gFW)	No. Internodes	Length of the roots (cm)	No. of roots	Rooting (%)
0	0	4.08±0.57^{a}	3.80±1.39^{b}	1.63±0.15^{b}	0.36±0.20^{ab}	11.00±1.63^{a}	0.10±0.25^{c}	0.60±1.57^{b}	26.6^{b}
0	10	3.40±0.25^{b}	3.30±1.49^{b}	1.20±0.04^{c}	0.29±0.13^{bc}	9.90±2.13^{a}	0.00^{d}	0.00^{c}	0.0^{d}
0	20	3.30±0.18^{b}	3.50±1.41^{b}	1.16±0.11^{d}	0.19±0.08^{c}	10.00±1.60^{a}	0.00^{d}	0.00^{c}	0.0^{d}
9.8	0	3.70±0.28^{a}	4.10±0.87^{b}	1.75±0.17^{a}	0.37±0.15^{a}	10.30±2.05^{a}	1.51±0.17^{a}	1.50±1.08^{a}	80.0^{a}
9.8	10	3.27±0.44^{b}	3.60±1.59^{b}	1.25±0.05^{e}	0.21±0.99^{bc}	8.80±2.26^{a}	0.88±1.06^{b}	0.50±0.53^{b}	53.0^{b}
9.8	20	3.30±0.54^{b}	2.60±1.61^{b}	1.10±0.08^{f}	0.33±0.22^{bc}	9.90±2.85^{a}	0.04±0.13^{c}	0.22±0.66^{b}	13.3^{c}
9.8 (Agar control)	40	3.65±0.57^{ab}	5.50±1.73^{a}	1.08±0.06^{f}	0.65±0.24^{a}	11.30±2.50^{a}	0.00^{d}	0.00^{c}	0.0^{d}

Different letters within a column indicate significant differences at $p \leq 0.05$ by Kruskal-Wallis/ Mann-Whitney test (n = 15). Values ± SD.

Afreen-Zobayed et al. (2000) report that the photoautotrophic culture of sweet potato (*Ipomoea batata* L. (Lam)) significantly stimulated the growth of the leaves (leaf area) using vermiculite as substrate with respect to the control in agar. Also, Iarema et al. (2012) noted that the photoautotrophic conditions developed for the micropropagation of the Brazilian ginseg (*Pfaffia glometata* (Spreng.) Pedersen) appears to increase the leaf area of *in vitro* plants using culture medium solidified with agar. In *Limonium* spp. plants, Lian et al. (2002) report that growth in photoautotrophic conditions, the growth of the surface area of the leaf and the number had a superior effect. In *Doritaenopsis* orchid under photoautotrophic culture conditions and with increased CO_2 in the culture vessel, also achieved the best results with respect to heterotrophic culture conditions for the variables leaf area and length (Shin et al., 2013). This was also observed in the present study on papaya. However, photoautotrophic culture conditions are not also suitable for the growth of some *in vitro* plants cultured as in the case of coconut (*Cocos nucifera*

L.), where without the presence of sucrose in the culture medium, plants had a smaller leaf area with respect to those grown with sucrose (Fuentes et al., 2005).

In the treatment without sucrose and with IBA after 17 days of culture, 50% of the papaya plants rooted, and the evaluation done at 37 days it reached 80.0%. Also, the treatment with 10 g L^{-1} of sucrose and IBA, similar results were obtained, but with lower values in all the evaluations done (Table 2). The rooting percentage previously expressed, reiterates the value of this auxin as an inducer of the process with enhanced effects through the lack of or low levels of sucrose in the culture medium. In addition, the results of this study suggest that continuous exposure to the auxin (IBA) improves the frequency of root formation, which is observed in the results obtained at 17, 27 and 37 days of culture (Tables 2, 3, 4 and Figure 1F). Plants grown without sucrose and with IBA also obtained the highest values in the variables height and fresh weight at 37 days of culture (Table 4). This behaviour is good evidence of the effectiveness of the culture conditions and zeolite as a substrate, ventilation of the culture vessels, IBA as a rooting inducer, and the absence of sucrose probably favored the source-demand effect so that photoautotroph would enhance the rooting stimulus of auxin. Several authors note the superior effect that IBA has with respect to other auxins for rooting of *in vitro* shoots in papaya (Yu et al., 2000; Teixeira da Silva et al., 2007; Kumar et al., 2012; Nzilani et al., 2013; Sekeli et al., 2013). These last authors reported that the best results in the rooting of *in vitro* shoots of transgenic papaya, Eksotika variety were achieved when first placing the shoots in culture medium with 9.8 µM IBA and in darkness for 4 days and thereafter were subcultured on MS culture medium at 50% of salt concentration, 10 µM of riboflavin and vermiculite as support.

According to George (2008), IBA easily becomes IAA causing a slow release of IAA and thus providing a continuous supply of the most common active auxin in concentrations, which may be more suitable for rooting. Calamar and de Klerk (2002) report the interaction between sucrose and auxin in the rooting of apple shoots. Increasing the sucrose concentration shifted the dose-response curve of auxin. The auxin concentration at the maximum response obtained, indicates that the cells require more auxin to give the response and / or that less free auxin reaches the target cells. There are reasons to believe that sucrose enhances the sensitivity to auxin. The results obtained in this study are contrary to reported by these authors. For sucrose concentrations studied (0, 10 and 20 g L^{-1}) without auxin rooting percentages of papaya shoots were very low or zero. However in the presence of auxin (IBA) root formation was achieved, but the percentage of rooting were decreasing in the middle that sucrose concentration was increased. It seems that for these photoautotrophic culture conditions attached to the zeolite as porous support had a positive effect on the rooting of shoots than in mixotrophic culture condition with 10 and 20 g L^{-1} of sucrose. About it, Malamy and Ryan (2001) reported that when *Arabidopsis* seedlings are grown on nutrient media with a high sucrose to nitrogen ratio, lateral root initiation is dramatically repressed. Auxin localization appears to be a key factor in this nutrient-mediated repression of lateral root initiation. They isolated a mutant, *lateral root initiation 1* (*lin1*) that overcomes the repressive conditions. This mutant produces a highly branched root system on media with high sucrose to nitrogen ratios. The *lin1* phenotype is specific to these growth conditions, suggesting that the *lin1* gene is involved in coordinating lateral root initiation with nutritional cues.

Fujiwara and Kozai (1995) report that the use of porous support material improves the environment in the root zone and thereby increase rooting. A high porosity of the culture medium increases the concentration of oxygen around the rooting system, enhancing root development and improving water and nutrient absorption by the *in vitro* plants. In addition, the extensive root system produced *in vitro,* appears to contribute to the higher percentage of plant survival during acclimatization to greenhouse conditions and in the field. The results described in this study may be related to the characteristics conferred by the zeolite. According to Flores et al. (2007), zeolite drastically reduces the leaching of potassium cations (K +) and ammonium (NH_4+), also it facilitates solubilization of phosphate by the available phosphorus to plants and therefore stimulates radical development. Zeolite is a crystalline hydrated aluminum silicate with three-dimensional structures, characterized by the ability to hold and release water and exchange ions without modifying their atomic structure, exchange cations such as Ca_2+, Mg_2+, K+ and NH_4+; and various phosphate compounds, ammonium and organic matter components. It has a rigid three-dimensional structure formed by a network of interconnected tunnels creating a large surface area for the cation exchange and moisture adsorption. Similar results in terms of correlation between improved root system, improved growth and high survival rate were obtained in other plant species such as acacia (*Acacia mangium*) (Ermayanti et al., 1999); coffee (*C. arabusta*) (Nguyen et al., 1999); sweet potatoes (*I. batata* L. Lam.) (Afreen-Zobayed et al., 1999); *Eucalyptus* sp. (Zobayed et al., 2001); four Australian papaya varieties (Kaity et al., 2009) and in the variety Eksotika (Sekeli et al., 2013); Orchid (*Doritaenopsis sp.*) (Shin et al., 2013) using different types of porous materials (vermiculite, perlite and mixtures of both). This response was also reached in this work in papaya plants using the porous zeolite mineral as substrate. The presence of contaminants (bacteria and fungi) in all the treatments was quantified (Table 5). It is noteworthy that despite the increased exchange through the lid of the culture vessel, the visual presence of contamination in the treatments without sucrose was 0% for the total time of the experiment of 37

Table 5. Contamination in the culture vessel with increased ventilation at different days of culture during rooting and *in vitro* acclimatization of papaya plants.

Treatment		Percentage contamination (%)		
IBA(µM)	Sucrose (g L^{-1})	17 days	27 days	37 days
0	0	0	0	0
0	10	0	0	100
0	20	0	0	100
9.8	0	0	0	0
9.8	10	0	0	80
9.8	20	0	0	100
9.8 (Agar control)	40	0	0	0

Table 6. Effects of the interaction sucrose and IBA on the concentration of chlorophyll a and b and total carotenoids content in the leaves of *in vitro* papaya (*Carica papaya* L. var. Red Maradol) plants in culture vessels with increased ventilation and zeolite as a support at 17 days of culture.

IBA (µM)	Sucrose (g L^{-1})	Chlorophyll a (mg g^{-1}FW)	Chlorophyll b (mg g^{-1}FW)	Carotenoids (mg g^{-1}FW)
0	0	0.842 ±0.07^{a}	0.519±0.04^{a}	0.397±0.03^{a}
0	10	0.407±0.17^{a}	0.343±0.14^{a}	0.296±0.05^{a}
0	20	0.554±0.03^{a}	0.526±0.02^{a}	0.358±0.00^{a}
9.8	0	0.578±0.15^{a}	0.551±0.10^{a}	0.311±0.07^{a}
9.8	10	0.464±0.14^{a}	0.449±0.13^{a}	0.266±0.08^{a}
9.8	20	0.765±0.08^{a}	0.682±0.15^{a}	0.319±0.07^{a}
9.8 (Agar control)	40	0.599±0.07^{a}	0.471±0.70^{a}	0.286±0.14^{a}

Different letters within a column indicate significant differences at $p \leq 0.05$ by Tukey test (n=15). Values ± SD.

days. The treatments with sucrose showed no contamination during the assessments done at 17 and 27 days, but at 37 days, which shows the level of air cleanliness in the culture chamber and that the conditions and management developed can be used for further scaling.

Physiological parameters: Chlorophyll a, b and carotenoids

In terms of the quality of *in vitro* plants, the effects of the factors involved in the management of the contents of the active pigments in the photosynthetic process are analyzed. No significant differences were noticed for the variables chlorophyll a, chlorophyll b and carotenoids for the factors auxin and sucrose for *in vitro* shoots of papaya at 17 days of culture between the different treatments and the control (Table 6); significant differences were however observed in the assessments at 27 and 37 days as shown in Figures 2 and 3. At 27 days of culture there was a significant interaction between auxin and sucrose factors influencing the variable chlorophyll a. In Figure 2A as seen, when there was no sucrose in the culture medium without auxin, the plant produces significantly more chlorophyll a. Also, at 27 days there was a significant interaction between auxin and sucrose factors influencing the response of the variable chlorophyll b. As shown in Figure 2B when no sucrose was added to the culture medium, the plant produces significantly more chlorophyll b when there was no auxin than when the medium was supplemented with it. In the presence of sucrose, production levels of chlorophyll a and b decreased independently of the presence of auxin. With increasing levels of sucrose in the culture medium, production levels of chlorophyll a and b remain low regardless of the presence or absence of auxin. The response is quite similar for both molecules in this species. There was no interaction between the factors sucrose and auxin, only that sucrose was significant, influencing the response of the variable carotenoids content. When no sucrose was added to the culture medium, the plant produces significantly more carotenoids. As long as there is sucrose in the culture medium, this production was reduced significantly, although between the concentrations between 10 and 20 g L^{-1} of sucrose this decrease is not significant (Figure 2C).

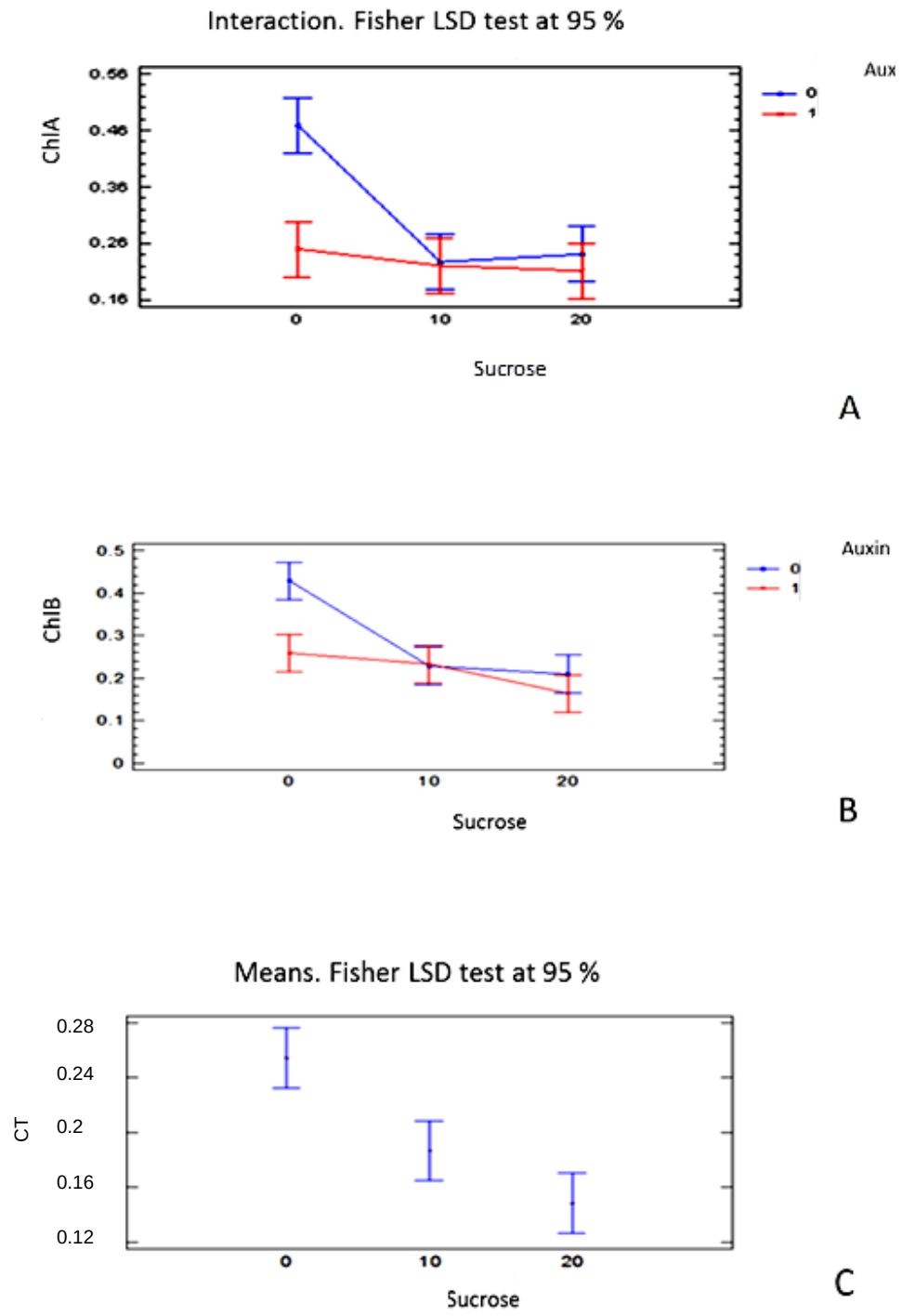

Figure 2. Effect of sucrose and IBA on the concentration of chlorophyll a, b, and total carotenoids content in the leave of *in vitro* papaya (*Carica papaya* L. var. Red Maradol) plants growing in culture vessel with increased ventilation and zeolite as support at 27 days de culture (A) Chlorophyll a (B) Chlorophyll b and (C) Carotenoids content. Reference: (0) without IBA and (1) 9.8 µM of IBA. Statistical difference between means according to the Fisher LSD test at $p \leq 0.05$.

At 37 days of culture, there was a significant interaction between the factors sucrose and auxin influencing the response of the variable chlorophyll a (Figure 3A). When there is no sucrose in the culture medium, the plant produces significantly more chlorophyll a than when there is no auxin. In the presence of sucrose, the production

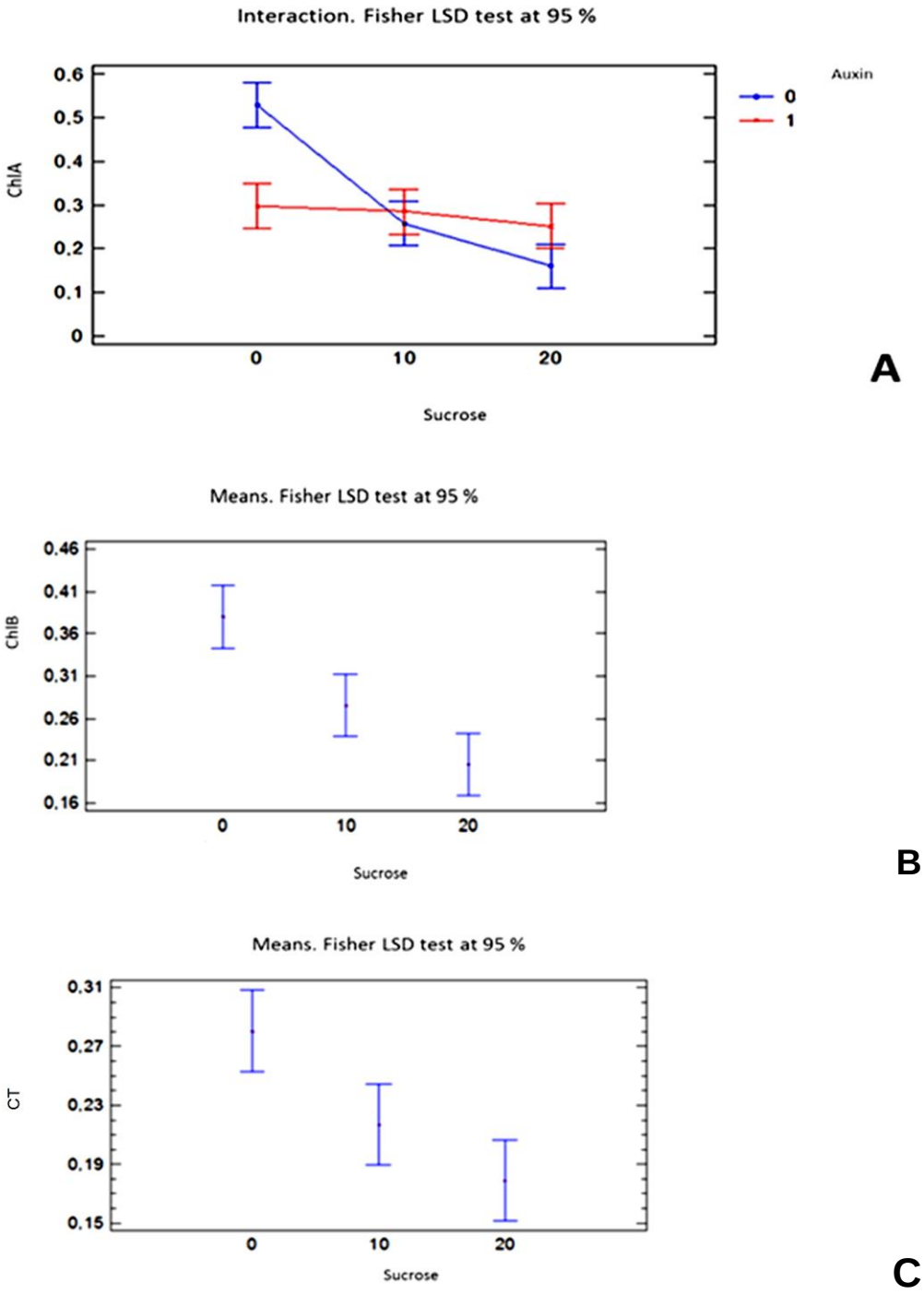

Figure 3. Effect of sucrose and IBA on the content of chlorophyll a, b, and total carotenoids content in the leave of *in vitro* papaya (*Carica papaya* L. var. Red Maradol) plants growing in culture vessel with increased ventilation and zeolite as support at 37 days of culture (A) chlorophyll a (B) chlorophyll b and (C) carotenoids content. Reference: (0) without IBA and (1) 9.8 µM of IBA. Statistical difference between means according to the Fisher LSD test at $p \leq 0.05$.

levels of chlorophyll a decreased regardless of the presence of auxin, but this decrease is significant in the case that there is no auxin for the greater concentration for sucrose, without differences in the case of 10 g L^{-1}. Sucrose alone had a significant influence on the content of chlorophyll b (Figure 3B). When there was no sucrose

Table 7. Effects of the interaction sucrose AIB on the photosynthetic activity (μmol CO_2 $m^{-2}s^{-1}$) and transpiration (mmol H_2O $m^{-2}s^{-1}$) in *in vitro* papaya plants (*Carica papaya* L. var. Red Maradol) cultured in culture vessels with increased ventilation and zeolite as a support at 17 days of culture.

IBA (μM)	Sucrose (g L^{-1})	Photosynthesis (μmol CO_2 $m^{-2}s^{-1}$)	Transpiration (mmol H_2O $m^{-2}s^{-1}$)
0	0	8.360±3.05^{ab}	11.221±1.05^{a}
0	10	3.766±1.56^{b}	3.335±0.81^{e}
0	20	3.611±1.30^{b}	2.767±1.28^{f}
9.8	0	8.892±1.47^{a}	4.701±0.91^{c}
9.8	10	8.957±1.30^{a}	3.881±0.73^{d}
9.8	20	8.716±1.65^{a}	1.936±1.07^{g}
9.8 (Agar control)	40	3.643±2.75^{b}	8.194±1.56^{b}

Different letters within a column indicate significant differences at $p \leq 0.05$ by Tukey test (n=15). Values ± SD.

added to the culture medium, the plants produced significantly more chlorophyll b. As long as there is sucrose in the culture medium, this production decreased, without being significant between the concentrations of 10 and 20 g L^{-1} sucrose. Sucrose alone had a significant influence on chlorophyll b at 37 days of culture. It is observed that when there is no sucrose in the culture medium, the plant produces significantly more chlorophyll b. Sucrose also had a significant effect influencing the variable carotenoids. When there was no sucrose in the culture medium, the plant produces significantly more carotenoids (Figure 3C). As long as there is the presence of sucrose in the culture medium, the production of these chlorophyll pigments decreased significantly, even though between the two concentrations of sucrose this decrease is not significant.

Physiological parameters: Photosynthesis and transpiration

Best photosynthetic values were achieved when the *in vitro* shoots were grown in culture medium with auxin and different concentrations of sucrose, even though they were also high in the treatment without the presence of IBA and without sucrose at 17 days of culture. In photomixotrophic culture conditions, transpiration levels were low with respect to the heterotrophic and photoautotrophic conditions. This is due to the presence of sucrose in the culture medium which the plant used as an energy source and it was not required for an increase in the photosynthetic activity and thus the opening and closing of the stomata, which made the transpiration levels so low. Nevertheless, the lowest levels of transpiration was obtained in plants grown in 20 g L^{-1} sucrose since the osmotic potential of the culture medium was higher, therefore for *in vitro* plants it is more difficult to take up water and hence transpiration rate was lower. For photoautotrophic conditions in the absence of the auxin, the plants did not have any roots at 17 days of culture, which resulted in a high rate of photosynthesis, but also high transpiration and having no roots to take up water for photosynthesis they had to have a greater stomata activity for the intake of CO_2, causing a greater transpiration (Table 7).

Plants grown in photomixotrophic conditions and without auxin, presented the lowest values of photosynthesis. In this regard, Rolland et al. (2002), Amiard et al. (2005); Jo et al. (2009) refer to plants that were grown in culture medium with sucrose, exhibited reduced photosynthesis, probably due to the presence of a sufficient energy source (sugar) and other metabolic activities. Franck et al. (2006) reported that sucrose plays a central role in the mechanism mediating control of the regulation by decreasing photosynthesis. The low rate of substrate regeneration for the carboxylation of ribulose bisphosphate (RuBP) due to the accumulation of soluble sugars in the leaves is the possible result in the inhibition of photosynthesis (Azcon-Bieto, 1983).

However, results obtained by these authors indicate that a greater amount of starch granules found in the chloroplasts of leaves of plants grown in the greenhouse probably were part of the storage product. On the contrary, in *in vitro* seedlings they did not show any starch granules, probably because the rate of photosynthesis is low or exogenous sucrose caused a negative feedback on the enzyme level of the plastid for starch biosynthesis (Krapp and Stitt, 1994). However, plants grown in photomixotrophic conditions and auxin, had high photosynthetic rate equal to those grown in photoautotrophic conditions, this might be because these plants began to develop their rooting system, which offset the loss of water for photosynthesis, making efficient use of water (Table 7).

Photosynthesis in plants grown on agar (heterotrophic control) was very low compared with their high transpiration rate, a reason that adds to the justification for the zero survival assessed at 17 days after planting in the acclimatization phase. This demonstrates the low ability to control water loss of these plants in heterotrophic

culture conditions. The levels of photosynthetic pigments also corresponded with this result given their involvement in the photosynthetic process. When sucrose was zero, the contents of carotenoids and chlorophylls were high and also appear to achieve good performances of the collecting antennas and the light producing complexes which integrate the photosystems involved and which constitute the pigments analyzed and other components. Although, the content of chlorophyll is not a direct indicator related to the photosynthetic capacity (Fujiwara et al., 1992); this is a good indicator of the state of the photosynthetic apparatus (Seon et al., 2000). This happened when the *in vitro* papaya plants were evaluated at 17 days of culture in the different treatments with and without photoautotrophic culture conditions where there were no significant differences observed among them; however, when determining the photosynthetic rate, there were significant differences between the different treatments as shown in Table 7. In this regard, Iarema et al. (2012) obtained the same response on analyzing the content of chlorophyll pigments and carotenoids of *in vitro* plants of Brazilian ginseg [*P. glometata* (Spreng.) Pedersen] cultured in the absence of sucrose and in the culture vessel with greatest level of exchange or ventilation and hence had an increase in the photosynthetic activity.

However, other authors reported the increase of photosynthetic pigments and increased photosynthetic activity in *in vitro* shoot cultured of *Limonium* spp. (Lian et al., 2002) and in *Dendrobium candidum* Wall. ex Lind *(*Xiao et al., 2007). The results obtained in this study support those reported by Kozai and Kubota (2005) on the benefits of photoautotrophic micropropagation over conventional micropropagation. The benefits from a biological point of view include: (1) promoting growth and photosynthesis; (2) high rates of survival and a smooth transition to environmental conditions *ex vitro*; (3) elimination of morphological and physiological disorders; (4) no callus formation at the base of the explant and (5) less plant lost due to contamination by microorganisms.

Ex vitro acclimatization

The treatment without sucrose and 9.8 µM IBA reached the highest percentage of survival which are suitable for rooting percentage that had *in vitro* papaya plants at 17 days of culture (Table 2 and Figure 1F). The treatments with the presence of auxin had the highest percentages of rooting, which corresponded to those of the greatest survival. The problem of very low survival is confirmed if appropriate management strategies are not performed that guarantee better quality of *in vitro* plants, with emphasis on their rooting pattern, and treatments without IBA at 17 days of culture (Table 2). Afreen-Zobayed et al. (2000) report that, in sweet potato 90% achieved survival of plants cultured *in vitro* in photoautotrophic conditions compared to 73% of those grown on agar. Kozai et al. (2005) reported in the species Calla Lily (*Zantedeschia elliottian* L.), 95% survival (photoautotrophic conditions) at 12 days after transplanting to acclimatization phase relative to 60% of plants grown in heterotrophic conditions. Also, in the species China fir (*Cunninghamia lanceolata* (Lambert) Hooker) only 16% survival in *in vitro* plants cultured in heterotrophic conditions was obtained and 95% in photoautotrophic. However, Jo et al. (2009) report that the best results in *ex vitro* acclimatization was reached for *Alocasia amazonica* plants cultured with 3.0% sucrose and not those that were cultured in autotrophic conditions.

Conclusion

The management of papaya plants var. Red Maradol obtained through somatic embryogenesis during the transition *in vitro-ex vitro* integrated by using zeolite as a support, the combination of zero or low levels of sucrose, increased ventilation and use of auxin IBA (9.8 µM) as an inducer of rooting, improve the quality of the plants and thus their survival.

Conflict of interests

The authors did not declare any conflict of interest.

ACKNOWLEDGEMENTS

We are also grateful to MD. Terrence Gilliard, from Biotechnology Lab. of Saint Lucia Island for his kind help in revising the English language.

REFERENCES

Afreen-Zobayed F, Zobayed SMA, Kubota C, Kozai T, Hasegawa O (1999). Supporting material affects the growth and development of in vitro sweet potato plantlets cultured photoautotrophically. *In vitro* Cell Dev. Biol. Plant 35:470-474.

Afreen-Zobayed F, Zobayed SMA, Kubota C, Kozai T, Hasegawa O (2000). A combination of vermiculite and paper pulp supporting material for the photoautotrophic micropropagation of sweet potato. Plant Sci. 157:225-231.

Amiard V, Mueh KE, Demmig-Adams B, Ebbert V, Turgeon R, Adams WW III (2005). Anatomical and photosynthetic acclimation to light environment in species with differing mechanisms of phloem loading. Proc. Natl. Acad. Sci. USA. 102: 12968-12973.

Azcón-Bieto J (1983). Inhibition of photosynthesis by carbohydrates in wheat leaves. Plant Physiol. 73:681-686.

Badr A, Angers P, Desjardins Y (2011). Metabolic profiling of photoautotrophic and photomixotrophic potato plantlets (Solanum tuberosum) provides new insights into acclimatization. Plant Cell Tiss. Organ Cult. 107:13-24.

Brainerd KE, Fuchigami LH, Kwiatkowski S, Clark CS (1981). Leaf anatomy and water stress of aseptically cultured Pixy plum grown under different environments. HortScience 16:173-175.

Calamar A, de Klerk GJ (2002). Effect of sucrose on adventitious root

regeneration in apple. Plant Cell Tissue Organ Cult. 70: 207-212.
Cardona AC, Araméndiz TH, Barrera CC (2009). Estimation of leaf area of papaya (*Carica papaya* L.) based on non-destructive sampling. Actualidad y Divulgación Científica Journal 12: 131-139.
Chen MH, Chen CC, Wang DN, Chen FC (1991). Somatic embryogenesis and plant regeneration from immature embryos of *Carica papaya* x *Carica cauliflora* cultured *in vitro*. Can. J. Bot. 69: 1913-1918.
Dhekney SA, Litz RE, Moraga D, Yadav A (2007). Is it possible to induce cold tolerance in papaya through genetic transformation? Acta Hortic. 738:159-164.
Ermayanti TM, Imelda M, Tajuddin T, Kubota C, Kozai T (1999). Growth promotion by controlling the *in vitro* environment in the micropropagation of tropical plant species. In: Proceeding of International workshop on conservation and sustainable use of tropical bioresources, Nov. 9-10, Tokyo, Japan. pp.10-25.
FAOSTAT (2014). Food and Agriculture Organization of the United Nations Database. In: http://www.apps.fao.org [Consulted: December 5, 2014].
Fitch M, Manshardt R (1990). Somatic embryogenesis and plant regeneration from immature zygotic embryos of papaya (*Carica papaya* L.). Plant Cell Rep. 9:320-324.
Flores MA, Galvis S, Hernández M, De León G, Payán Z (2007). Effect of addition of zeolite (clinoptilolite and mordenite) in a Andosol chemical environment on edaphic and oats growth. Interciencia J. 32(10): 692-696.
Franck N, Vaast P, Génard M, Dauzat J (2006). Soluble sugars mediate sink feedback down-regulation of leaf photosynthesis in field-grown *Coffea arabica*. Tree Physiol. 26:517-525.
Fuentes G, Talavera C, Oropeza C, Desjardins Y, Santamaría JM (2005). Exogenous sucrose can decrease *in vitro* photosynthesis but improve field survival and growth of coconut (*Cocos nucifera* L.) *in vitro* plantlets. *In vitro* Cell. Dev. Biol. Plant. 41:69-76.
Fujiwara K, Kira S, Kozai T (1992). Time course of CO_2 exchange of potato cultures *in vitro* with different sucrose concentrations in the culture medium. J. Agric. Meteorol. 48: 49-52.
Fujiwara K, Kozai T (1995). Physical microenvironment and its effects In: Aitken-Christie J, Kozai T, Smith Mal (eds) Automation and environmental control in plant tissue culture. Springer Publishers, Dordrecht. pp. 319-369.
George EF (2008). Plant propagation by tissue culture, 3rd edition, volI. The background. In: George EF, Hall MA, De Klerk G-J (eds) Plant tissue culture procedure-background. Springer, Dordrecht, pp. 1-28.
Gribaudo I, Novello V, Restagno M (2001). Improved control of water loss from micropropagated grapevines (*Vitis vinifera* cv. Nebbiolo). VITIS 40(3):137-140.
Iarema L, da Cruz ACF, Saldanha CW, Dias LLC, Vieira RF, de Oliveira EJ, Otoni WC (2012). Photoautotrophic propagation of Brazilian ginseg [*Pfaffia glometata* (Spreng.) Pedersen]. Plant Cell Tissue Organ Cult. 110(3): 227-238.
Jo E-A, Kumar RT, Hahn E-J, Paek K-Y (2009). *In vitro* sucrose concentration affects growth and acclimatization of *Alocasia amazonica* plantlets. Plant Cell Tissue Organ Cult. 96:307-315.
Kadleček P, Tichá I, Haisel D, Čapková V, Schäfer Ch (2001). Importance of *in vitro* pretreatment for *ex vitro* acclimatization and growth. Plant Sci. 161: 695-701.
Kaity A, Parisi AM, Ashmore SA, Dew RA (2009). Root initiation and acclimatization of papaya plants. In: Proc. IIIrd IS on Acclim. and Establt. of Micropropagated plants, Romano A (ed). Acta Hort. 812:387-392.
Kozai T (2010). Photoautotrophic micropropagation-environmental control for promoting photosynthesis. Propag. Ornam. Plants 10:188-204.
Kozai T, Kubota C (2005). Concepts, definitions, ventilation methods, advantages and disadvantages. In: Kozai T, Afreen F, Zobayed SMA (eds) Photoautotrophic (sugar-free medium) micropropagation as a new propagation and transplant production system. Springer, Dordrecht, pp. 19-30.
Kozai T, Xiao Y,Nguyen QT, Afreen F, Zobayed SMA (2005). Photoautotrophic (sugar-free medium) micropropagation system for large-scale commercialization. Propag. Ornam. Plants 5:23-34.
Krapp A, Stitt M (1994). Influence of high carbohydrate content on the activity of plastidic and cytosolic isoenzyme pairs in photosynthesis tissues. Plant Cell Environ. 17:861-866.
Kumar PR, Kumar SR, Lokman HM (2012). Propagation of papaya (*Carica papaya* L.) cv. Shahi through *in vitro* culture. Bangladesh J. Bot. 41: 191-195.
Lian ML, Murhty HN, Paek KY (2002). Culture method and photosynthetic photon flux affect photosynthesis, growth and survival of *Limonium* Misty Blue *in vitro*. Sci. Hortic. 95:239-249.
Malabadi RB, Kumar SV, Mulgund GS, Nataraja K (2011). Induction of somatic embryogenesis in papaya (*Carica papaya*). Res. Biotechnol. 2(5):40-55.
Malamy JE, Ryan KS (2001). Environmental regulation of lateral root initiation in *Arabidopsis*. Plant Physiol. 127(3): 899-909.
Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
Nguyen TQ, Kozai T, Nguyen KL, Nguyen UV (1999). Effects of sucrose concentration, supporting material and number of air exchanges of the vessel on the growth of *in vitro* coffee plantlets. Plant Cell Tissue Organ Cult. 58:51-57.
Norikane A, Takamura T, Morokuma M, Tanaka M (2010). *In vitro* growth and single-leaf photosynthetic response of *Cymbidium* plantlets to super-elevated CO_2 under cold cathode fluorescent lamps. Plant Cell Rep. 29: 273-283.
Nzilani NM, Karambu FR, Edward GM, Wanjiru AK (2013). *In vitro* regeneration of selected Kenyan papaya (*Carica papaya* L.) lines through shoot tip culture. Afr. J. Biotechnol. 12(49):6826-6832.
Pierik R (1990). Rejuvenation and micropropagation. In: Nijkamp H, Van dar Plas, Van ArtrijiK J (eds): Progress in Plant Cellular and Molecular Biology. Springer, Dordrecht. pp. 91-101.
Posada-Pérez L, Gómez-Kosky R, Reyes VM (2007). Somatic embryogenesis in *Carica papaya* L. var. Red Maradol. Biotecnología vegetal J. 7(3): 131-138.
Rolland F, Moore B, Sheen J (2002). Sugar sensing and signaling in plants. Plant Cell 14: 185-205.
Sekeli R, Abdullah JO, Namasivayam P, Muda P, Abu Bakar UM (2013). Better rooting procedure to enhance survival rate of field grown Malaysian Eksotika papaya transformed with 1-Aminocyclopropane-1-carboxylic acid oxidase gene. ISRN Biotechnology 13: 1-10.
Seon J, Cui Y, Kozait T, Paek K (2000). Influence of *in vitro* growth conditions on photosynthetic competence and survival ration of *Rehmannia glutinosa* plantlets during acclimatization period. Plant Cell Tissue Organ Cult. 64: 135-142.
Shin K-S, Park S-Y, Paek K-Y (2013). Sugar metabolism, photosynthesis, and growth of in vitro plantlets of *Doritaenopsis* under controlled microenvironmental conditions. *In vitro* Cell. Dev. Biol. Plant 49: 445-454.
Stirban M (1985). Procese primare în fotosintezã. Didact Ed. Si Pedag. Bucharest. p. 229.
Teixeira da Silva JA (2014). Photoauto-,Photohetero-and Photomixotrophic *in vitro* propagation of papaya (*Carica papaya* L.) and response of seed and seedlings to light-emitting diodes. Thammasat Int. J. Sci. Technol. 19(1):57-71.
Teixeira da Silva JA, Giang DTT, Chan M-T, Sanjaya, NA, Chai M-L, Chico-Ruíz J, Penna S, Granstrom T, Tanaka M (2007). The influence of different carbon sources, photohetero-, photoauto and photomixotrophic conditions on protocorm-like body organogenesis and callus formation in thin cell layer culture of hybrid *Cymbidium* (Orchidaceae). Orchid Sci. Biotechnol. 1(1-2):15-23.
Xiao Y, Niu G, Kozai T (2011). Development and application of photoautotrophic micropropagation plant system. Plant Cell Tissue Organ Cult. 105:149-158.
Xiao Y, Zhang Y, Dang K, Wang D (2007). Growth and photosynthesis of Dendrobium candidum plantlets cultured photoautotrophically. Propag. Ornam. Plants 7: 86-96.
Yu TA, Yeh SD, Cheng YH, Yang JS (2000). Efficient rooting for establishment of papaya plantlets by micropropagation. Plant Cell Tissue Organ Cult. 61: 29-35.
Zhang M, Zhao D, Ma Z, Li X, Xiao Y (2009). Growth and photosynthetic capability of *Momordica grosvenori* plantlets grown photoautotrophically in response to light intensity. HortScience. 44(3):757-763.

Zobayed SMA, Afreen F, Kozai T (2001). Physiology of eucalyptus plantlets cultured photoautotrophically under forced ventilation. *In vitro Cell* Dev. Biol. Plant 37:807-813.
Zobayed SMA, Afreen F, Xiao Y, Kozai T (2004). Recent advancement in research on photoautotrophic micropropagation using large culture vessels with forced ventilation. *In vitro* Cell. Dev. Biol. Plant 40: 450-458.