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In Vitro PROPAGATION OF *Moringa oleifera* LAM. CULTIVARS

Propagación *in vitro* de cultivares de *Moringa oleifera* Lam.

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ABSTRACT. Moringa has leaves, flowers and fruit with a high level of vitamins, minerals and proteins of interest for the food and medical industries. This species can be propagated by seeds or cuttings, methods that not suitable to introduce new cultivars. The aim of this research was to establish an *in vitro* propagation protocol of different cultivars of *Moringa oleifera* Lam. To disinfect seeds three different disinfection times were evaluated five, seven and nine minutes in 10 gL^{-1} sodium hipoclorite. Two methods for i*n vitro* micropropagation were used: conventional system (semi-solid culture medium) and temporary immersion bioreactor. In the *in vitro* rooting phase, four concentrations of the naphthalene acetic acid and Indole butyric acid auxins (0; 2,5; 5,0 and 7,5 µmol L^{-1}) were evaluated and also the kind of explant. The survival rates of the sprout during the acclimatization phase was evaluated in relation to their origin in the rooting phase. Besides the effect of the genotype on *in vitro* establishment and multiplication was determined. The disinfection of the Supergenius cultivar was better at seven minutes of treatment with sodium hipoclorite, with 54 % of germination. The Temporary Immersion Bioreactor increased the sprouts morphological quality and multiplication coefficient from 6,2 until 16,1 of the Supergenius cultivar. Rooting was successful without auxin and acclimatization survival was around 85 % at 35 days for shoots rooted without growth regulators, regardless of explant origin. It was shown that the genotype influenced the establishment and multiplication of explants.

Key words: cultivars, protocol, plant growth regulators

RESUMEN. La Moringa posee hojas, flores y frutos con un alto contenido de vitaminas, minerales y proteínas de interés para las industrias alimenticias y médicas. Su reproducción es por semillas y estacas, métodos insuficientes si se desean introducir nuevos cultivares. El presente trabajo tuvo como objetivo establecer un protocolo para la propagación *in vitro* de *Moringa oleifera* Lam. La desinfección de las estructuras embrión-endospermo con hipoclorito de sodio 1 % (V:V) se realizó a los cinco, siete y nueve minutos. La multiplicación *in vitro* se realizó por: sistemas convencionales (medio de cultivo semi-sólido) y biorreactores de inmersión temporal. En el enraizamiento *in vitro* se evaluó la concentración del ácido naftalen acético y el ácido indol butírico (0; 2,5; 5,0 y 7,5 µmol L^{-1}) y el tipo de explante. Se evaluó la supervivencia de los brotes en la aclimatización teniendo en cuenta la procedencia de la fase de enraizamiento. Se determinó el efecto del genotipo en el establecimiento y la multiplicación *in vitro*. La desinfección del cultivar Supergenius fue mejor a los siete minutos, con un porcentaje de germinación del 54 %. Los biorreactores de inmersión temporal incrementaron la calidad morfológica de los brotes y el coeficiente de multiplicación aumentó de 6,2 a 16,1 para el cultivar Supergenius. El enraizamiento se logró sin auxinas y la supervivencia en aclimatización fue de un 85 % a los 35 días para los brotes que se enraizaron sin reguladores del crecimiento, independientemente del explante de procedencia. Se demostró que el genotipo influyó en el establecimiento y multiplicación de los mismos.

Palabras clave: cultivares, protocolo, reguladores del crecimiento vegetal

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INTRODUCTION

Moringa oleifera Lam is a tree of the family *Moringaceae* (1). This species is native to the area of India, Afghanistan, Pakistan (2). Because of its high resistance to stress conditions, it is now widespread in much of the world, including America (3). This plant is a natural resource that has great utility as a food supplement rich in vitamins, antioxidants, oils, microelements and proteins (2). In addition to the benefit to the food industry, in traditional medicine is used to prevent pathologies associated with the lack of essential elements of the diet, such as childhood blindness (4). It is also attributed uses in the purification of water (5) and in the production of biodiesel (6). Some authors also assign bio-stimulant activity to the growth of some cultures. Recently it has been reported in the literature the obtaining of natural compounds from this plant for agricultural and medical use (5, 7). Due to these properties, the cultivation of this species has increased in recent years (5). Traditional methods such as seeding of seeds and cuttings have been the most used to satisfy the consumption / demand relationship (8). However, time and money investments in pruning, field preparation, planting and harvesting are large and sometimes limit the potential for use.

In recent years, *in vitro* cultivation techniques have been used in order to obtain more plants of this species and save material resources. However, there are few reports that refer to it. This creates the need to deepen the research concerning this tree of great importance in food security, health and agriculture. Temporary Immersion Bioreactors (BIT®) have been successfully used for the *in vitro* propagation of plants (9, 10). These semi-automated systems with the use of the liquid medium favor *in vitro* nutrition, as well as the renewal of gases within the culture flask, which increases the growth and development of the sprouts (9, 11). In addition, significant improvements are obtained in the morpho-physiological indicators of the outbreaks that guarantee greater survival and growth of the same in *ex vitro* conditions (12). The present work aimed to establish a protocol for the *in vitro* propagation of *Moringa oleifera* Lam.

MATERIALS AND METHODS

Vegetal material

Fruits of seven cultivars of *Moringa oleifera* Lam (Supergenius, Criolla Ciego de Avila, PKM-2, Paraguay, Guatemala, Plain and Criolla Holguín) were collected in different areas of the national territory. To obtain the seeds, the fruits were dried at room temperature for a week. The seeds were removed from the tegument, the embryo-endosperm structures were used, and those with a diameter of 0,8 mm and 1 cm were selected; washed with commercial detergent for five minutes and rinsed three times with copious running water.

Effect of the disinfection time of the *Moringa oleifera* Lam seeds cv Supergenius

Disinfection of the explants (embryo-endosperm structures) was performed with sodium hypochlorite 1 % (V: V) under agitation for five, seven and nine minutes. Subsequently, 10 mL of culture medium, which was composed of 100 % MS salts (14), supplemented with 20 g L^{-1} sucrose, 1 mg L^{-1} of thiamine, 100 Mg L^{-1} of myo-inositol and without growth regulators. The pH was adjusted to 5,7. The culture conditions were of a photoperiod of 16 light hours and eight hours of darkness and a stream of photosynthetically active photons of 37 µmol $m^{-2}s^{-1}$ (FAITHFUL digital photometer model FT-710) at a temperature of 26 ± 1 °C . 48 explants per treatment were used. After seven days of culture the number of contaminated explants was determined. At 21 days, the number of germinated and non-germinated embryo-endosperm structures was evaluated. The results were expressed in percentage.

In vitro multiplication of sprouts of *Moringa oleifera* Lam cv Supergenius

From the germinated embryo-endosperm structures, two types of explants were obtained: apices (stem segment of approximately 3 cm with a pair of leaves) and nodal segments (stem segment of approximately 3 cm with two nodes and two leaves). All explants were placed in a semi-solid culture medium containing 100 % MS (13) salts, supplemented with 30 g L^{-1} sucrose, 1 mg L^{-1} of thiamine, 100 mg L^{-1} Myo-inositol and without growth regulators. The pH was adjusted to 5,7. The inoculum density was seven explants per flask and the volume of culture medium was 25 mL. Culture conditions were maintained as in the previous experiment. The explants were subcultured three times every 21 days, maintaining the same composition of the culture medium.

[A] Yasmeen, A. *Exploring the potential of moringa (Moringa oleifera) leaf extract as natural plant growth enhancer* [en línea]. Ph. D. thesis, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan, 2011, [Consultado: 10 de febrero de 2016], Disponible en: <http://moringatrees.org/moringa-doc/moringa-natural-plant-growth-enhancer.pdf>.

[B] Artiga, S. M. E. *Efecto del BAP y 2,4-D en la inducción in vitro de tejido callogénico a partir de láminas foliares, segmentos peciolares y vitro-explantes hipocotiledores y radiculares de Moringa oleífera* [en línea]. Tesis de Grado, Escuela Agrícola Panamericana, Zamorano, Honduras, 2012, 25 p., [Consultado: 1 de febrero de 2016], Disponible en: <http://bdigital.zamorano.edu/handle/11036/1057>.

Subsequently, ten stem segments of approximately 3 cm in length were taken with two nodes and two leaves from the conventional multiplication and placed in Temporary Immersion Bioreactors (BIT®) (14) containing 800 mL of liquid culture medium with equal Composition than the previous one. Three dives were performed per day and the immersion time was four minutes. Three BITs were used and each explant constituted an experimental unit. Culture conditions were maintained as in the previous experiment. After 21 days of cultivation, the number of sprouts per explant, length of shoot, number of internodes, number of roots, stem thickness, presence of hyperhidric sprouts, and the multiplication coefficient were calculated as the quotient of the number of final outbreaks and the number of initial explants.

In vitro rooting of _Moringa oleifera_ Lam sprouts cv Supergenius

For *in vitro* rooting, a semi-solid culture medium composed of the MS salts (13), supplemented with 40 g L^{-1} sucrose, 1 mg L^{-1} thiamine, 100 mg L^{-1} of Myo-inositol and growth regulators (ANA) and indole butyric acid (AIB) in various concentrations (0; 2,5; 5,0 and 7,5 µmol L^{-1}). The pH was adjusted to 5,7. Two types of explants were used, the bases of stems of approximately 3 cm in length with two knots and two leaves and the segment of stem of approximately 3 cm in length, with two knots and two leaves. The inoculum density was seven explants per flask and the volume of culture medium was 25 mL. The culture conditions described above were maintained. 30 explants per treatment were used. Each explant constituted an experimental unit. At 21 days of cultivation, the indicators of shoot height, number of internodes, and number of roots, length and thickness of the most extensive root were evaluated.

Acclimatization of plants of _Moringa oleifera_ Lam cv Supergenius

The sprouts of approximately 5 cm in length, with four leaves and presence of roots from the different *in vitro* rooting treatments, were placed in peat tablets (jiffys). The culture conditions were 80 ± 3 % relative humidity and 25 ± 2 °C temperature (Thermo Hygrometer digital TECPEL® model DTM-303), photoperiod corresponding to the cycle of the day (16 hours light/8 hours darkness) with a photosynthetically active photon flux of 608 µmol $m^{-2}s^{-1}$ and atmospheric conditions of CO_2 concentration between 375 and 400 µmol mol^{-1} at 12:00 pm. Irrigation was performed with watering can once a day in the hours between 9:30 am and 11:00 am. Thirty plants were used per treatment. Each plant constituted an experimental unit. At 7, 14, 21, 28 and 35 days of culture the survival percentage was calculated according to the formula:

% survival = (number of alive plants / total of plants) x 100

Effect of genotype on seed germination of _Moringa oleifera_ Lam

The seeds of the seven cultivars of *Moringa oleifera* Lam (Supergenius, Criolla Ciego Ávila, PKM-2, Paraguay, Guatemala, Plain and Criolla Holguín) were disinfected with 1 % sodium hypochlorite (V: V) Under stirring for seven minutes. The cultivation conditions described for the germination of the cultivar Supergenius were maintained. 48 embryo-endosperm structures were used per cultivar. After seven days of culture the number of contaminated explants was determined. At 21 days, the number of germinated and non-germinated embryo-endosperm structures was evaluated. The results were expressed as a percentage.

Effect of genotype on _Moringa oleifera_ Lam multiplication

In order to evaluate the effect of the genotype in the multiplication, it proceeded in the same way as with the cultivar Supergenius previously described. After 21 days of BIT cultivation, the number of sprouts per explant, shoot length, number of internodes, root presence, stem thickness and the presence of hyperhidric sprouts were evaluated. In addition, the multiplication coefficient was calculated as the quotient between the number of final sprouts and the number of initial explants.

Statistical analysis

The SPSS utility (15), the analysis of simple, bifactorial and three-factor variance (ANOVA) with different levels was used in the statistical processing of the data. The means of the treatments were compared using the Tukey multiple rank test ($p < 0.05$). In some cases it was necessary to transform the data to achieve the assumptions of the parametric tests performed.

RESULTS AND DISCUSSION

Effect of the disinfection time of the seeds of *Moringa oleifera* Lam cv Supergenius
The effect of the disinfection time with 1% sodium hypochlorite (V: V) on the percentage of contaminated, germinated and non-germinated embryo-endosperm structures during *in vitro* establishment is shown in Table I. The lowest percentage of contamination was obtained when the disinfection was carried out during nine minutes, with significant differences of the rest of the treatments. The highest percentage of germination corresponded to the treatment in which

sodium hypochlorite 1 % (V: V) was used for seven minutes, which differed significantly from treatments of five and nine minutes. It was also observed that with increasing disinfection time, the percentage of non-germinated seeds increased even though the percentage of contamination was lower. This behavior could be related to a phytotoxic effect of sodium hypochlorite (16). From these results the time of seven minutes as the one suitable for the disinfection of the moringa seeds was selected.

In vitro multiplication of *Moringa oleifera* Lam sprouts cv Supergenius

In the multiplication of *M. oleifera* Lam. cv Supergenius in the conventional system a high multiplication coefficient (6,2) was obtained despite not using growth regulators in the culture medium. This could be associated mainly with the juvenility of the plant material and the rupture of the apical dominance that favored the activation of the axillary sprouting as well as to a high endogenous content of phytohormones such as zeatin A (17), which could favor the formation of axillary sprouts. The use of BIT increased the multiplication coefficient up to 16,1 (Table II), demonstrating the positive effect of this cropping system on the multiplication of *M. oleifera* Lam. cv Supergenius. In addition, the morphological indicators evaluated showed favorable results. The use of the liquid culture medium and the renewal of the internal gas atmosphere of the culture vessel favored the incorporation and assimilation of the nutrients, increasing the growth and accumulation of biomass (14, 18).

In vitro rooting of *Moringa oleifera* Lam sprouts cv Supergenius

The effect of the concentration of two auxins (ANA and AIB) and the type of explant on morphological indicators in the *in vitro* rooting phase of *Moringa oleifera* Lam sprouts. cv. Supergenius are shown in Table III. The number of roots was higher when segments of stems with 5,0 $\mu molL^{-1}$ of ANA and 7,5 $\mu molL^{-1}$ of IBA were used as explant, with no significant differences between them.

Table I. Effect of the disinfection time of the embryo-endosperm structures of *Moringa oleifera* Lam cv Supergenius

Time (min)	Contaminated (%)	Germinated (%)	Ungerminated (%)
5	39,55 c	31,35 b	29,10 b
7	24,96 b	54,24 a	20,80 c
9	18,00 a	36,00 b	46,00 a

Means with different letters represent significant statistical differences ($p \leq 0.05$) (ANOVA one factor, Tukey) (n = 48)
For statistical treatment, the data were transformed according to x'= 2arcoseno $(x / 100)^{0.5}$

Table II. Morphological indicators of Moringa oleifera Lam cv Supergenius after multiplication in BIT. Each value represents the mean of 30 repetitions

Sprout length (cm)	Number of internodes	Number of roots	Stem thickness (mm)	Number of hyperhidric sprouts	CM
4,9	4,2	1,9	3,1	0	16,1

Table III. Effect of the application of ANA and AIB in the *in vitro* rooting phase of *Moringa oleifera* Lam sprouts cv Supergenius

Auxin	Concentration ($\mu mol\ L^{-1}$)	Type of explant	Height (cm)	Number of internodes	Number of roots	Longer root length (cm)	Larger root thickness (mm)
Control	0,0	Base	5,16 b	2,66 b	3,50 d	3,97 a	0,13 b
		Stem segment	5,33 b	3,73 a	3,80 d	4,24 a	0,10 b
	2,5	Base	6,63 a	2,50 b	6,06 bc	3,60 ab	0,24 a
		Stem segment	3,04 d	1,36 c	8,36 b	3,10 b	0,14 b
ANA	5,0	Base	4,18 c	1,86 bc	8,73 b	3,27 b	0,12 b
		Stem segment	2,55 d	1,03 e	11,30 a	2,12 c	0,10 b
	7,5	Base	2,82 d	1,33 d	6,67 bc	3,57 b	0,11 b
		Stem segment	2,64 d	1,10 e	7,36 b	3,49 b	0,11 b
	2,5	Base	5,42 b	2,36 b	3,80 d	3,60 b	0,10 b
		Stem segment	3,80 cd	1,93 bc	4,70 cd	3,26 b	0,11 b
AIB	5,0	Base	4,90 bc	2,40 b	3,20 d	2,95 c	0,10 b
		Stem segment	4,25 c	2,00 b	5,20 c	3,50 b	0,10 b
	7,5	Base	5,60 b	2,90 b	7,90 b	3,20 b	0,10 b
		Stem segment	5,76 b	2,43 b	11,80 a	3,18 b	0,10 b

Means with different letters represent significant statistical differences ($p \leq 0.05$) (ANOVA trifactorial, Tukey) (n = 30). For thestatistical treatment, thedataof number ofinternodes and number ofroots were transformed according to x'= x0,5 and x'=(x + 0,5) 0,5, respectively

The length of the most extensive root was greater in the control, regardless of the type of explant, without significant differences with the treatment in which stem bases were used with 2.5 µmol L^{-1} of ANA. This treatment also showed the best result in terms of the thickness of the root and the sprout height, with significant differences in the rest of the treatments. The number of internodes was higher for the stem segments of the control treatment, with significant differences with the rest of the treatments. In the explants coming from the base of the stalk the callus formation was favored in the basal region, which affected the formation of roots. This could be related to the endogenous increase of auxin concentration, since it is recognized that Moringa has a high content of IAA and the exogenous addition of ANA and AIB could provoke uncontrolled cell division giving rise to callous mass (19). This condition in the explants is undesired since the roots that form from this structure are of low quality (20).

Acclimatization of *Moringa oleifera* Lam sprouts cv Supergenius

Table IV shows the application effect of the auxins (ANA and AIB) during *in vitro* rooting on the survival percentage of the shoots in the acclimatization phase. The use of auxins in the *in vitro* rooting phase, regardless of type and concentration, markedly affected the survival of sprouts during the course of time. The control treatment, where no auxins were used for rooting, showed the best survival values at all evaluated moments, regardless of the explant type used. After 35 days of culture, survival was greater than 85 % for the control treatment. For the treatments where auxins were used the survival values at the end of this phase were very low ranging from 0 to 6,6 %. The observed behavior could be associated to the fact that the roots formed *in vitro* are not very functional and with few adsorbent hairs, which does not guarantee the nutrition of the sprouts in the initial stage of acclimatization (21). In addition, in some plants, *in vitro* roots are lost during transplantation to the substrate, so during this process the plants have to adapt to new growing conditions and face the stress conditions to which they are exposed during transit *In vitro-ex-vitro* could affect survival percentage. The application of auxins promoted the rooting of the sprouts; However, in the control treatment the roots that were formed are more functional, which guaranteed the survival during the acclimatization. This could be associated to the fact that the auxin supplement favors callus formation at the base of sprouts that impedes the functioning of the roots in *ex vitro* conditions affecting the survival of the plants.

Effect of genotype on seed germination of *Moringa oleifera* Lam

Table V shows the effect of disinfection for seven minutes with 1 % of sodium hypochlorite (V: V) on the percentage of contaminated, germinated and non-germinated embryo-endosperm structures during the *in vitro* establishment of *M. oleifera* Lam cv Paraguay, Plain, PKM-2, Guatemala, Creole Ciego Avila, Creole Holguin. For the cultivars evaluated, the percentage of contamination was lower than that of the cultivar Supergenius, with the exception of the cultivar Criolla Holguín, which showed a percentage of contamination of 95,80 %. The highest percentage of germination was obtained in the cultivars Criolla Ciego Ávila and Plain, without significant differences between them. These values of germination were superior to that obtained with the cultivar Supergenius. The germination of the rest of the cultivars was low. The cultivar Criolla Holguín was severely affected by the contamination and non-germination of the embryo-endosperm structure, so it was not possible to continue the experimentation with this cultivar.

Table IV. Percentage survival of *Moringa oleifera* Lam cv Supergenius sprouts in the acclimatization phase

Auxin	Concentration (mg L^{-1})	Explant	Survival (%)				
			7 days	14 days	21 days	28 days	35 days
Control	0,0	Base	100,0 a	96,0 a	93,0 a	90,0 a	90,0 a
		Segment of stem	96,0 a	93,0 a	93,0 a	86,0 a	86,0 a
ANA	2,5	Base	73,0 bc	50,0 c	26,0 b	6,6 b	6,6 b
		Segment of stem	73,0 bc	46,0 d	20,0 c	0,0 c	0,0 c
	5,0	Base	73,0 bc	60,0 b	26,0 b	3,3 bc	3,3 b
		Segment of stem	66,0 c	50,0 c	23,0 bc	10,0 b	6,6 b
	7,5	Base	70,0 bc	53,0 bc	26,0 b	3,3 bc	0,0 c
		Segment of stem	73,0 bc	60,0 b	30,0 b	10,0 b	6,6 b
AIB	2,5	Base	80,0 b	60,0 b	23,0 bc	10,0 b	6,6 b
		Segment of stem	73,0 bc	63,0 b	26,0 b	6,6 b	6,6 b
	5,0	Base	66,0 c	50,0 c	26,0 b	0,0 c	0,0 c
		Segment of stem	86,0 b	53,0 bc	30,0 b	0,0 c	0,0 c
	7,5	Base	76,0 b	56,0 bc	20,0 c	6,6 b	0,0 c
		Segment of stem	73,0 bc	60,0 b	16 c	10,0 b	3,3 b

Means with different letters represent statistically significant differences at each evaluation time ($p \leq 0.05$)
(ANOVA three factor, Tukey) (n = 30). For statistical treatment, the data were transformed according to $x' = 2\arcsin(x / 100)^{0.5}$

Table V. Effect of the disinfection time of the embryo-endosperm structures in different cultivars of *Moringa oleifera* Lam

Cultivars	Contaminated (%)	Germinated (%)	Ungerminated (%)
Paraguay	6,25 b	6,25 c	87,50 c
Plain	6,25 b	87,50 a	6,25 a
PKM-2	6,25 b	6,25 c	87,50 c
Guatemala	2,08 a	6,25 c	91,60 c
Criolla Ciego Ávila	8,30 b	91,60 a	0,00 a
Criolla Holguín	95,80 d	0,00 d	4,20 a
Supergenius	24,96 c	54,24 b	20,80 b

Percentages with different letters represent significant statistical differences (p≤0.05) (ANOVA one factor, Tukey) (n = 48)
For statistical treatment, the data were transformed according to x'= 2arcoseno (x / 100) $^{0.5}$

Effect of genotype on *Moringa oleifera* Lam multiplication

The values of the multiplication coefficient in conventional system of the cultivars of *M. oleifera* Lam (Paraguay, Plain, PKM-2, Guatemala and Criolla Ciego Ávila) is shown in Table VI. The best results were obtained with the cultivars Supergenius, Plain and Paraguay, without statistically significant differences among them. The lowest results were obtained with the cultivar Criolla Ciego Ávila, despite being the one that showed the best results in terms of germination of the embryonic structures with 91,60 %. The values of the morphological indicators evaluated after the multiplication in BIT of *M. oleifera* Lam are shown in Table VII. For the length indicator of the outbreak, the best results were obtained for the cultivars Supergenius, Paraguay and Plain, with no statistical differences among them. On the other hand, the number of internodes was superior in the cultivars Supergenius, Paraguay and PKM-2, without statistical differences among them.

Table VI. Coefficient of multiplication in conventional system of different cultivars of Moringa oleifera Lam

Cultivars	CM
Paraguay	5,8 a
Plain	6,0 a
PKM-2	5,3 b
Guatemala	4,8 b
Criolla Ciego Ávila	3,9 c
Supergenius	6,2 a

Means with different letters represent statistically significant differences (p≤0.05) (ANOVA one factor, Tukey) (n = 30)

Root formation was evident in all cultivars except Paraguay and Criolla Ciego Ávila, with the highest number of roots in the cultivars Supergenius and PKM-2, with no significant differences amng them. The stem thickness indicator did not show significant differences among the cultivars evaluated. Also, no hyperhidric sprouts occurred in any of the cultivars. As for the multiplication coefficient, the best result corresponded to the cultivar Supergenius, followed by the cultivar Paraguay. For these cultivars, the BIT increased by more than twice the multiplication coefficient, while for the rest of the cultivars this indicator did not have a significant increase over the conventional system. The results demonstrated the genotype effect on the germination of the embryo endosperm structures and the *in vitro* multiplication. The observed differences among the cultivars could be related to the physiological and sanitary state of the mother plant and the seeds, as well as the size, age and time of taking the explants. In addition, it is known that the genotype response depends on the gene expression of each individual, which depends on the environment where the individual develops (22).

CONCLUSIONS

- A protocol for the *in vitro* propagation of cultivars of *Moringa oleifera* Lam was obtained, which was characterized by its effectiveness in the number and quality of shoots. It was characterized by the absence of growth regulators during the establishment, multiplication and rooting of shoots,

Table VII. Morphological indicators of shoots of *Moringa oleifera* Lam different cultivars of. During multiplication in BIT

Cultivars	Sprout length (cm)	Number of internodes	Number of roots	Stem thinckness (mm)	CM
Paraguay	4,7 a	3,9 a	0,0 d	3,0 a	12,9 b
Plain	4,4 ab	3,2 b	0,6 c	3,2 a	7,5 c
PKM-2	3,6 c	3,5 ab	1,9 a	3,0 a	5,2 e
Guatemala	4,2 b	2,9 c	1,0 b	3,0 a	6,7 d
Criolla Ciego Ávila	3,5 c	3,4 b	0,0 d	3,0 a	5,1 e
Supergenius	4,9 a	4,2 a	1,9 a	3,1 a	16,1 a

Means with different letters for each column represent significant differences (p≤0.05) (ANOVA one factor, Tukey) (n = 30)
For the statistical treatment, the data of number of internodes were transformed according to x'= x0,5 and for the number of roots and with x'= (x + 0,5) $^{0.5}$

which had a positive impact on the high survival rates achieved during acclimatization.

- With the technique use of temporary immersion it was possible to double the multiplication rate in some cultivars demonstrating the effectiveness of this method in the propagation of this plant. It is evident that the endogenous contents of auxins and cytokinins in this plant favored the *in vitro* propagation, so that the obtaining of natural extracts and their evaluation in the *in vitro* morphogenesis of other plants are of great interest and they are aspects to be addressed in future investigations.

BIBLIOGRAPHY

1. Bonal, R. R.; Rivera, O. R. M. y Bolívar, C. M. E. ‘‘*Moringa oleifera*: una opción saludable para el bienestar’’. *MEDISAN*, vol. 16, no. 10, octubre de 2012, pp. 1596-1599, ISSN 1029-3019.
2. Villarreal, G. A.; Angulo, O. y Johana, K. ‘‘Review of characteristics and uses of the plant *Moringa oleifera*’’. *Investigación y Desarrollo*, vol. 22, no. 2, julio de 2014, pp. 309-330, ISSN 0121-3261.
3. del Toro, M. J. J.; Carballo, H. A. y Rocha, R. L. ‘‘Valoración de las Propiedades Nutricionales de *Moringa Oleífera* en el Departamento de Bolívar’’. *Revista de Ciencias*, vol. 15, 13 de febrero de 2012, pp. 23-30, ISSN 0121-1935.
4. Posmontier, B. ‘‘The Medicinal Qualities of *Moringa Oleifera*’’. *Holistic Nursing Practice*, vol. 25, no. 2, 2011, pp. 80-87, ISSN 0887-9311, DOI 10.1097/HNP.0b013e31820dbb27.
5. Martín, C.; Martín, G.; García, A.; Fernández, T.; Hernández, E. y Puls, J. ‘‘Potenciales aplicaciones de *Moringa oleifera*. Una revisión crítica’’. *Pastos y Forrajes*, vol. 36, no. 2, junio de 2013, pp. 137-149, ISSN 0864-0394.
6. Olson, M. E. y Fahey, J. W. ‘‘*Moringa oleifera*: un árbol multiusos para las zonas tropicales secas’’. *Revista mexicana de biodiversidad*, vol. 82, no. 4, diciembre de 2011, pp. 1071-1082, ISSN 1870-3453.
7. Yasmeen, A.; Basra, S. M. A.; Wahid, A.; Nouman, W. y Rehman, H. U. ‘‘Exploring the potential of *Moringa oleifera* leaf extract (MLE) as a seed priming agent in improving wheat performance’’. *TURKISH JOURNAL OF BOTANY*, vol. 37, no. 3, 3 de junio de 2013, pp. 512-520, ISSN 1300-008X.
8. Phiri, C. y Mbewe, D. N. ‘‘Influence of *Moringa oleifera* leaf extracts on germination and seedling survival of three common legumes’’. *International Journal of Agriculture and Biology*, vol. 12, no. 2, 2010, pp. 315-317, ISSN 1560-8530, CABDirect2.
9. Escalona, M. ‘‘Temporary immersion beats traditional techniques on all fronts’’. *Prophyta Annual*, 2006, pp. 48–50, ISSN 0921-5506, 0921-5506.
10. Watt, M. P. ‘‘The status of temporary immersion system (TIS) technology for plant micropropagation’’. *African Journal of Biotechnology*, vol. 11, no. 76, 20 de septiembre de 2012, pp. 14025-14035, ISSN 1684-5315, DOI 10.5897/AJB12.1693.
11. Peñate, L.; Concepción, O.; Aragón, C.; Rodríguez, R.; González Olmedo, J. L.; Escalona, M.; Cid, M. y Pina, D. ‘‘Evaluación del efecto de tres condiciones de cultivo *in vitro* en la calidad de plántulas de caña de azúcar propagadas en Biorreactores de Inmersión Temporal’’. *Biotecnología vegetal*, vol. 7, no. 3, 2007, pp. 161-169, ISSN 1609-1841.
12. Aragón, C.; Carvalho, L.; González, J.; Escalona, M. y Amancio, S. ‘‘The physiology of ex vitro pineapple (*Ananas comosus* L. Merr. var MD-2) as CAM or C3 is regulated by the environmental conditions’’. *Plant Cell Reports*, vol. 31, no. 4, 2 de diciembre de 2011, pp. 757-769, ISSN 0721-7714, 1432-203X, DOI 10.1007/s00299-011-1195-7.
13. Murashige, T. y Skoog, F. ‘‘A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures’’. *Physiologia Plantarum*, vol. 15, no. 3, 1 de julio de 1962, pp. 473-497, ISSN 1399-3054, DOI 10.1111/j.1399-3054.1962.tb08052.x.
14. Escalona, M.; Lorenzo, J. C.; González, B.; Daquinta, M.; González, J. L.; Desjardins, Y. y Borroto, C. G. ‘‘Pineapple (*Ananas comosus* L. Merr) micropropagation in temporary immersion systems’’. *Plant Cell Reports*, vol. 18, no. 9, mayo de 1999, pp. 743-748, ISSN 0721-7714, 1432-203X, DOI 10.1007/s002990050653.
15. Pérez, L. C. *Técnicas estadísticas con SPSS 12: aplicaciones al análisis de datos*. edit. Pearson Prentice Hall, 2005, 824 p., ISBN 978-84-205-4410-6.
16. García, M. B.; Abeal, E. E.; Rodríguez, I. P. y Rodríguez, S. M. ‘‘Uso de distintos tratamientos de desinfección en el cultivo *in vitro* de *Dioscorea alata* L. clon caraqueño’’. *Revista Colombiana de Biotecnología*, vol. 11, no. 2, 15 de octubre de 2009, pp. 127-135, ISSN 1909-8758.
17. de Feria, M.; Chávez, M.; Barbón, R.; La O, M.; Pérez, M.; Jiménez-Terry, F.; Quiala, E. y Agramonte, D. ‘‘Multiplicación in vitro de plantas de *Pinus caribaea* var. caribaea’’. *Biotecnología Vegetal*, vol. 9, no. 4, 2009, pp. 217-224, ISSN 1609-1841.
18. Jiménez, E.; Reyes, C.; Machado, P.; Pérez Alonso, N.; Capote, A.; Pérez, A. y Eichler Loebermann, B. ‘‘Multiplicación *in vitro* de *Morinda royoc* L. en Sistemas de Inmersión Temporal’’. *Biotecnología vegetal*, vol. 11, no. 2, 2011, pp. 115-118, ISSN 1609-1841.
19. Guillén, S.; Martínez-Palacios, A.; Martínez, H. y Martínez-Ávalos, J. G. ‘‘Organogénesis y embriogénesis somática de *Beaucarnea inermis* (Asparagaceae), una especie amenazada del noreste de México’’. *Botanical Sciences*, vol. 93, no. 2, 2015, pp. 221-230, ISSN 2007-4298, DOI 10.17129/botsci.129.

20. Rodríguez, B. M.; Carrillo, L. R.; Chacón, F. M.; Hormazábal, V. N.; Tampe, P. J. y Tighe, N. R. ‘‘Enraizamiento in vitro y ex vitro de microtallos de Ugni molinae Turcz., una especie nativa de Chile’’. *Gayana. Botánica*, vol. 72, no. 1, junio de 2015, pp. 14-20, ISSN 0717-6643, DOI 10.4067 S0717-664 32015000100002.
21. García, A. V.; Sandrea, Y.; Gonzalez, O.; Diaz, A.; Albarran, J. G.; Schmidt, A.; Salazar, E.; Mujica, Y.; Casado, R.; Fernandez, J. y R, C. M. ‘‘Micropropagación de plantas de lechosa en recipientes de inmersión temporal a partir de brotes axilares’’. *Revista Colombiana de Biotecnología*, vol. 17, no. 1, 22 de mayo de 2015, pp. 70-78, ISSN 1909-8758, DOI 10.15446/rev.colomb.biote.v17n1.50718.
22. Orellana, P. P.; García, L.; Bermúdez, I.; Veitía, N. y Romero, C. ‘‘Manejo de hijos y ápices de cultivares de *Musa* spp. para iniciar la micropropagación y comportamiento durante seis subcultivos *in vitro*’’. *Biotecnología vegetal*, vol. 2, no. 2, 2002, pp. 77-81, ISSN 1609-1841.

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Note:

During the editing process it was not possible to access the work of retouching and improvement of images, so they have been inserted with the same quality as the ones sent by their authors.

The editorial

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