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ORIGINAL RESEARCH ARTICLE

In vitro establishment: *Monstera acuminata Koch* and *Monstera deliciosa* Liebm

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ABSTRACT

The study evaluated the aseptic establishment of *Monstera acuminata* Koch and *Monstera deliciosa* Liebm (Araceae) from leaves and the induction of *in vitro* organogenesis of *M. acuminata* K. from stem discs of young shoots. For this purpose, different disinfection protocols were applied to mature leaves and young shoots, from which leaf explants of approximately 1 cm^2 and stem discs of approximately 1 mm thickness were extracted. The explants were established in semi-solid media with different hormone treatments during the aseptic establishment stage and induction of organogenesis. Disinfection with 3% sodium hypochlorite (NaClO) for 20 min and 50% Murashige and Skoog[1] medium with plant tissue culture preservative (PPM) favored less oxidation in leaf explants of both species. All explants of *M. deliciosa* in both treatments grown in PPM-added medium and at different disinfection protocols survived, showed no contamination and more than 80% retained cellular activity up to 49 days of culture age. At 35 days of culture, with disinfection in Tween-20 + 20% ethanol + 2.5% NaClO, and seeding of explants in MS medium added with 1 mg/L of BAP, 0.5 mg/L of AIA and 0.1 mg/L of ANA, seven new shoots of stem discs were induced. *Monstera deliciosa* was more adaptable to *in vitro* conditions. Advances in aseptic establishment and induction of organogenesis in native Araceae for wicker production are the basis for *ex situ* conservation of local populations.

Keywords: Araceae; Aseptic Establishment; *Ex Situ* Conservation; Leaf Explants; Organogenesis

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1. Introduction

The conservation of wild populations of Araceae is important because fibers are obtained from them for various uses in Latin America. *Monstera deliciosa* Liebm. and *Monstera acuminata* Koch are two species of this plant family, useful for the construction of basketry tools, handicrafts and furniture, as well as having cultural and economic value in the Mam communities of the Tacaná Volcano, Chiapas, Mexico. Both species are typical elements of the Mesophilic Mountain Forest (MFM), a Mexican ecosystem threatened by deforestation[2]; therefore, due to their cultural and artisanal importance, they should be rescued under appropriate *ex-situ* conservation techniques.

In vitro culture is a possible technique that allows storing, selecting and maintaining germplasm under aseptic conditions, in order to introduce it back to its natural habitat[3]. In Araceae, this cultivation method has been little documented. Montes *et al.*[4] in juvenile leaves of *Anthurium andreanum* L. found that *in vitro* disinfection with double immersion in 0.53% NaClO + Tween-20 for 30 min, followed by another immersion in 0.27% NaClO + Tween-20 for 20 min, was efficient to ensure 96% of aseptic plant material; while Materán-Oviedo *et al.*[5]

recommend disinfection with 2.5% v/v NaClO for 15 min, followed by immersion in a 50 mg/L solution of benomyl + cysteine for 1 min.

Induction of organogenic callus from leaf explants has been possible in *Anthurium andreadrum* L., according to Atak and Celik[6] employing MS salts[1] with 50% salt concentration, supplemented with 0.6 mg/L 2,4-dichlorophenylacetic acid (2,4-D) and 1 mg/L benzyladenine (BA); while Valle-Sandoval *et al.*[7] recommend the use of MS salts[1], 3% sucrose and 0.6 % agar, in combination with benzylaminopurine (BAP) at 2 mg/L and indolacetic acid (IAA) of 0.1–1.8 mg/L or 0.1 mg/L of 2, 4-D and 1 mg/L of BA, for shoot induction from stem discs.

Due to the lack of *in vitro* disinfection and induction protocols for plants of the Araceae family of interest (*Monstera* sp., among them) the objective of this research was to establish protocols for the aseptic establishment and induction of *in vitro* organogenesis of *M. acuminata* Koch and *M. deliciosa* Liebm, from leaves and shoot stem discs.

2. Materials and methods

For the study, plants of *M. deliciosa* L. and *M. acuminata* K. were collected in communities of Soconusco (Chiapas, Mexico), respectively in Fracción Barrio Nuevo (N15°04.112", W92°06.740") and Guadalupe (N14°58.13", W92°18.117"). The collected cuttings were conserved in greenhouse conditions at 25 + 2 °C and 77% relative humidity for a period of 80 days in the facilities of the Rosario Izapa Experimental Field of the National Institute of Forestry, Agricultural and Livestock Research (CERI-INIFAP).

2.1 Aseptic establishment of *M. acuminata* and *M. deliciosa*

In this case, two mature leaves per species were used to obtain the explants. At the Plant Biotechnology Laboratory of CERI-INFAP, the leaves were washed with a solution of 5 ml of neutral soap in 200 ml of water. To obtain four 3 × 12 cm segments per leaf, the main rib was previously removed before disinfection.

The first disinfection protocol consisted of immersing the leaves in a fungicidal antioxidant solution under constant agitation composed of 30 g/L sucrose + 100 mg/L Meyer® ascorbic acid + 150 mg/L Meyer® citric acid + 1.5 g/L azoxystrobin (Amistar Sygenta®), for 15 min. Two washes were performed with sterile water; then they were immersed in a solution of ethanol CTR® at 70% for 15 s, then in a solution of 2.5% sodium hypochlorite (NaClO, commercial product Cloralex®) with three drops of Tween®-20 for 20 min under constant agitation. Subsequently, three washes were performed with sterilized distilled water for 2 min each and finally, they were placed for 5 min in the antioxidant solution, previously described.

The second disinfection protocol differed from the first, since the immersion in the fungicidal antioxidant solution was done under constant agitation for 3 min in a vacuum pump with double immersion in NaClO at 1.25% + three drops of Tween-20 for 15 min and in NaClO at 0.83% + three drops of Tween-20 for 10 min. Between each immersion, two washes of 2 min each were performed, and three washes with sterilized distilled water before immersion in the antioxidant solution.

Three different culture media were prepared. The first was the control medium consisting of Murashige and Skoog[1] culture medium with 50% of mineral salts, added with Gamborg vitamins[8], thiamine (0.4 mg/L), inositol (100 mg/l), sucrose (30 g/L), Phytagel Sigma® (3 g/L) and the growth regulators: 2,4-D acid Sigma® (1 mg/L) and BAP Sigma® (0.2 mg/L). The second consisted of the control medium added with the fungicide azoxystrobin Amistar® (1 g/L) and Polyvinylpyrrolidone (PVP) Sigma® (2 g/L); and the third was the control medium added with the agent Preservative for Plant Tissue Culture (PPM) Plant Cell Technology. Inc™ (1 mL/L). The culture media were sterilized at 120 °C and 101325 kg/cm^2 pressure for 20 min in autoclaves before being placed in a 60 × 15 mm Petri dish with 10 mL in a flow hood.

A total of 12 treatments were constructed with 20 replicates each (**Table 1**). Disinfected leaves were dissected into 1 cm^2 segments, each explant was placed in Petri dishes and incubated under dark conditions for 49 days. The variables evaluated

were survival percentage, contamination, cell activity and oxidation. The measurement of the survival, contamination and cell activity (growth) variables was binomial, with 1 (presence) and 0 (absence), while the oxidation variable was measured by the proportion of the explant surface darkened, with fixed percentages of 20, 25, 50, 50, 75 and 100%. Evaluations were made every 7 days until 49 days after sowing.

Table 1. Treatments for aseptic establishment of *M. acuminata* and *M. deliciosa*

Trat (No.)	Species		Disinfection protocol		Culture media		
	M. acuminata	***M. deliciosa***	**AFS + 70% Ethanol + 2.5% NaClO + AS**	**AFS VP + 70% Ethanol + 1.25% NaClO + 0.83% NaClO + AS**	**Half Control (HC)**	**HC + Amistar + PVP**	**HC + PPM**
1	+	-	+	-	-	+	-
2	+	-	+	-	-	-	+
3	+	-	+	-	+	-	-
4	+	-	-	+	-	+	-
5	+	-	-	+	-	-	+
6	+	-		+	+	-	-
7	-	+	+	-	-	+	-
8	-	+	+	-	-	-	+
9	-	+	+	-	+	-	-
10	-	+	-	+	-	+	-
11	-	+	-	+	-	-	+
12	-	+	-	+	+	-	-

Trat. = Treatment, AFS = Antioxidant fungicide solution, AFS VP = Antioxidant fungicide solution in vacuum pump, NaClO = sodium hypochlorite, AS = Antioxidant solution, HC = Half Control, PVP = Polyvinylpyrrolidone, PPM = Preservative for Plant Tissue Culture, + = Treatment applied.

2.2 Statistical analysis of data

The experiment consisted of a trifactorial design: 2 species, 2 disinfection protocols, 3 culture media. Statistical analysis of contamination, reactivation and oxidation responses was performed using a generalized linear model (GLM), with a binomial distribution for contamination and cell activity responses and normal distribution for oxidation. Multiple comparisons between treatments were performed with the application of orthogonal contrasts with Bonferroni correction and a significance level of 5%. Survival data were analyzed through a model with interval censoring, applying the asymptotic weighted log rank test with a significance level of 5% in a statistical program R version 2.3 for Windows[9].

2.3 Induction of organogenesis in *M. acuminata*

In the greenhouse, shoots of *M. acuminata* with an average length of 6.6 cm and a diameter of 4.5 mm were selected. The first disinfection protocol consisted of immersing the shoots in a 3% solution of Antibenzil Farmacéuticos Altamirano® surgical soap for 3 min; then in a 70% solution of Ethanol CTR® for 1 min, and subsequently in a 1.25% solution of NaClO Cloralex® + 0.5 mL of Tween-20, for 10 min, all under constant agitation. Three washes were performed with sterile distilled water for 3, 4 and 5 min and finally, the shoots were immersed in the antioxidant solution described above for 5 min[7].

In the second disinfection protocol, the explants were washed with tap water + 2 drops of Tween-20 for 5 min, then immersed in a 20% CTR® Ethanol solution for 10 s and washed with sterile water, then placed for 20 min in a 2.08% NaClO solution + 2 drops of Tween-20, under constant agitation. Three washes were performed with sterile distilled water for 3, 4 and 5 min and, finally, they were immersed in an antioxidant solution with benomyl Promyl® + Sigma® cysteine (1:1) at 50 mg/L for 5 min[5].

The culture media were prepared with Murashige and Skoog[1] salts, Gamborg vitamins[8], thiamine (0.4 mg/L), inositol (100 mg/L), sucrose

(30 g/L) and Bacto™ Agar BD (6 g/L). In the first culture medium, the growth regulators BAP Sigma® at 1 mg/L, AIA Sigma® at 0.5 mg/L, and naphthaleneacetic acid (ANA) Sigma® at 0.1 mg/L were added: In the second medium, BAP Sigma® at 2 mg/L and AIA Sigma® at 1 mg/L, and ANA Sigma® at 0.5 mg/L were added. The culture media were sterilized in autoclave at 120 °C and 101325 kg/cm^{-2} pressure for 20 min. In a laminar flow hood (Purifier® series, Labconco®) 10 ml were placed in a 60 × 15 mm Petri dish.

Shoots without enveloping leaves were cut into 1 mm thick discs (explant) under a Reichert® stereoscope. Sowing was done quickly to avoid tissue oxidation and the positive polarity of the discs was respected, according to the section of the apical, middle and basal shoots. One shoot was used for each treatment, and a total of four treatments with different replicates were established.

The explants of treatments 1 and 2 were subjected to the first disinfection protocol consisting of 3% soap + 70% ethanol + 1.25% NaClO + antioxidant solution, while those of treatments 3 and 4 were subjected to the second protocol (Tween-20 + 20% ethanol + 2.08% NaClO + benomyl solution + cysteine). The explants of treatments 1 and 3 were grown in medium supplemented with BAP (1 mg/L) + AIA (0.5 mg/L) + ANA (0.1 mg/L), and those of treatments 2 and 4 in medium supplemented with BAP (2 mg/L) + AIA (1 mg/L) + ANA (0.5 mg/L).

The treatments were maintained for 14 days in dark conditions at 28 °C and then in a 16-h photoperiod. The variables evaluated were survival percentage, contamination, shoot induction and callus formation, taking as measures values of 1 for presence and 0 for absence. The oxidation variable was measured with fixed percentages of 20, 25, 50, 50, 75 and 100%, according to the proportion of the explant surface showing response, and the number of new shoots per treatment. Evaluations were made every 7 days until day 35 after planting.

With the use of a Zeiss® stereoscope (1.0 X FWD 81 mm), freehand histological sections of the induced sprouts were carried out in order to know their cellular origin. The sections were stained with two drops of 0.05% fluoroglucinol for 2 min and one drop of 0.1 N hydrochloric acid (HCl) for 2 min, for observation under the Velab® light microscope at 4X, 10X and 40X.

Table 2. Effect of treatments on percent survival of mature leaf segments 49 days after planting. Averages of 20 replicates per treatment

Treatment	Survival (%)
T1. *M. acuminata* + 2.5% NaClO + MS (A + PVP)	43.30 b*
T2. *M. acuminata* + 2.5% NaClO + DM (PPM)	50.00 b
T3. *M. acuminata* + 2.5% NaClO + DM	40.00 b
T4. *M. acuminata* + 1.25% + 0.83% NaClO + MS (A + PVP)	45.00 b
T5. *M. acuminata* + 1.25% + 0.83% NaClO + MS (PPM)	45.00 b
T6. *M. acuminata* + 1.25% + 0.83% NaClO + DM	25.00 b
T7. *M. deliciosa* + 2.5% NaClO + MS (A + PVP)	95.00 a
T8. *M. deliciosa* + 2.5% NaClO + MS (PPM)	100.00 a
T9. *M. deliciosa* + 2.5% NaClO + DM	85.00 ab
T10. *M. deliciosa* + 1.25% +0.83% NaClO + MS (A + PVP)	90.00 a
T11. *M. deliciosa* + 1.25% + 0.83% NaClO + MS (PPM)	100.00 a
T12. *M. deliciosa* + 1.25% + 0.83% NaClO + DM	85.00 ab

*Values with equal letters are not statistically different (asymptotic weighted log rank, $P < 0.05$). NaOCl = sodium hypochlorite, MS = Murashige and Skoog[1] culture medium, A = Amistar, PVP = Polyvinylpyrrolidone, PPM = Preservative for Plant Tissue Culture.

2.4 Data analysis

The analysis of the response variables was descriptive. The percentages of oxidation, cellular activity and callus formation for each shoot section (apical, middle and basal) at 35 days after planting were plotted in the statistical program R version 2.3 for Windows.

3. Results

3.1 *In vitro* establishment of *M. acuminata* and *M. deliciosa*

In the aseptic establishment of leaf explants

in both species, differences ($P < 0.05$) were found between treatments for the survival variable ($x^2 = 77.6657$, gl = 11, P = 4.165e-12). Forty-nine days after planting, explants of *M. deliciosa* showed higher survival than those of *M. acuminata* (**Table 2**).

Statistical analysis of cell activity showed that the response or activity in explants had significant interaction between treatments ($x^2 = 48.082$, gl = 11, P = 1.38e-06) and time ($x^2 = 330.84$, gl = 1, P = 0.01). Cell activity consisted of folding, elongation, and swelling of leaf segments. Forty-nine days after sowing, only treatments 8, 10, 11 and 12 presented percentages higher than 80% of cell activity, followed by treatments 9 and 7; while in treatments 1, 2, 3 and 5 this activity varied between 60 and 40%, and in treatments 4 and 6 it was lower than 40% (**Figure 1**).

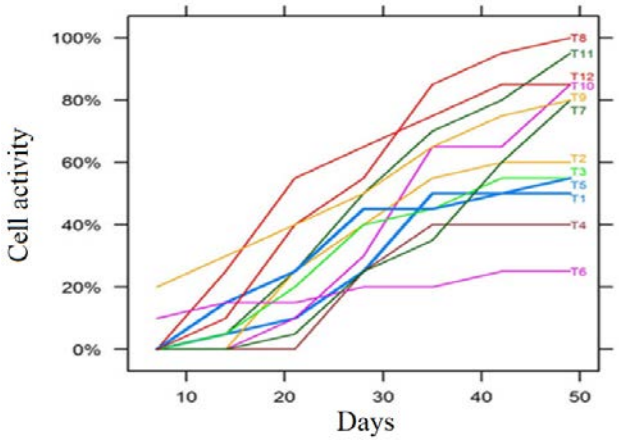

Figure 1. Cell activity curves of *M. deliciosa* and *M. acuminata* in the evaluated treatments.
T = Treatment.

In the analysis of variance of the logistic model of contamination, it was observed that culture time influenced explant contamination ($x^2 = 80.089$, gl = 1, P = 0.01) and at 49 days there were statistical differences between treatments ($x^2 = 23.694$, gl = 7, P = 1.29E-03).

Fungal contamination in explants was more frequent than bacterial contamination. *M. deliciosa* explants disinfected by the first protocol—fungicidal antioxidant solution + 70% Ethanol + 2.5% NaClO + Antioxidant solution—and seeded in culture medium with Amistar + PVP (treatment 7) and in medium with PPM (treatments 8); and treatment 11 of the second protocol—fungicidal antioxidant solution in vacuum pump + 70% Ethanol + 1.25% NaClO + 0.83% NaClO + Antioxidant solution—in culture medium with PPM did not present contamination.

Treatment 10—M. *deliciosa* + second disinfection protocol + MS medium (A + PVP)—showed a low response (5%). Treatments 9 and 12—*M. deliciosa* explants in control MS medium, but disinfected with different protocols—presented contamination percentages lower than 30% and were significantly different from treatment 4—*M. acuminata* + second disinfection protocol + MS medium (A + PVP)—with 55% and treatment 6—*M. acuminata* + second disinfection protocol + control MS medium—with 75%; while in the rest of the treatments more than half of the explants presented contamination levels between 50 and 55%).

Oxidation in the explants occurred from the third day after sowing. The analysis showed independent effects per factor, being highly significant ($P < 0.005$) the effects of species and culture medium ($P < 0.0001$) and significant ($P < 0.02$) for the disinfection protocol.

3.2 Induction of organogenesis in *M. acuminata*

Thirty-five days after sowing, explant survival in treatments 1 and 3—BAP (1 mg/L) + AIA (0.5 mg/L) + ANA (0.1 mg/L)—was greater than 80%, while in treatments 2 and 4—BAP (2 mg/L) + AIA (1 mg/L) + ANA (0.5 mg/L)—was lower.

The percentage of oxidation in the stem discs was higher in treatment 4 composed by the second disinfection protocol—ween 20 + 20 % ethanol + 2.08 % NaClO + benomyl + cysteine solution) and BAP (2 mg/L) + AIA (1 mg/L) + ANA (0.5 mg/L-followed by treatment 2—first disinfection protocol (3% soap + 70 % ethanol + 1.25 % NaClO + antioxidant solution) + BAP (2 mg/L) + AIA (1 mg/L) + ANA (0.5 mg/L—and by treatment 3—second disinfection protocol + BAP (2 mg/L) + AIA (1 mg/L) + ANA (0.5 mg/L) + ANA (0.5 mg/L).25 % + Antioxidant solution) + BAP (2 mg/L) + AIA (1 mg/L) + ANA (0.5 mg/L) and by treatment 3—second disinfection protocol + BAP (1 mg/L) +

AIA (0.5 mg/L) + ANA (0.1 mg/L) —while in treatment 1—first protocol + BAP (1 mg/L) + AIA (0.5 mg/L) + ANA (0.1 mg/L)—was less than 60%.

Table 3. Effects of species, disinfection protocol, and culture medium on oxidation percentages of mature leaf segments 49 days after planting. Averages of twenty replicates per treatment

Variable	Oxidation (%)
Species	
Monstera acuminata K. Koch.	73.36 ± 26.78† a*
Monstera deliciosa Liebm.	42.64 ± 18.54† b
Disinfection protocol	
SB + 2.5% NaClO	56.12 ± 26.59† a
CB + 1.25% + 0.83% NaClO	59.88 ± 28.62† b
Culture medium	
MS 50% + BAP/2,4-D + Amistar/PVP	63.85 ± 25.02† a
MS 50% + BAP/2,4-D	57.62 ± 29.86† b
MS 50% + BAP/2,4-D + PPM	52.53 ± 26.80† c

† Standard deviation.
SB = disinfection without vacuum pump,
CB = disinfection with vacuum pump,
NaClO = sodium hypochlorite,
MS 50% = Murashige and Skoog[1] 50% culture medium,
BAP = Benzylaminopurine,
2,4-D = 2,4-dichlorophenolacetic acid,
PPM = Preservative for Plant Tissue Culture,
PVP = P Polyvinylpyrrolidone,
Amistar = Systemic fungicide.
* Values with equal letters are not statistically different (Bonferroni correction, 0.05).

Stem discs showed bacterial contamination more frequently in basal explants. The explants of treatments 3 and 4 that were disinfected by the second disinfection protocol—Tween-20 + 20% ethanol + 2.08% NaClO + benomyl solution+ cysteine—showed percentages lower than 10%, while the explants of treatments 1 and 2 disinfected with the first protocol—3% soap + 70% ethanol + 1.25% NaClO + antioxidant solution—showed percentages higher than 30%, indicating that the second disinfection protocol was more efficient than the first (**Table 3**).

Treatments 2 and 3 presented percentages of cell activity higher than 80%. Treatment 3 presented a greater number of explants with new shoots, followed by treatments 1 and 4; while the explants of treatment 2 formed friable callus in greater numbers than the other treatments (**Table 3**).

4. Discussion

4.1 *In vitro* establishment of *M. acuminata* and *M. deliciosa*

The genotype and phenological stage of the plant material influenced the survival and cellular activity of the established explants. The leaf segments that were established came from mature tissue. The leaf laminae of *M. acuminata*, unlike those of *M. deliciosa*, presented the natural perforations of the species. These perforations in the leaf laminae of *Monstera* species are associated with early tissue senescence[10]. According to these authors, at the molecular level, the formation of perforations in the leaves of *M. obliqua* originates from the process of programmed cell death (PCD) early in leaf development. This interesting behavior in the morphology of Monstera leaves explains why the explants of *M. acuminata* survived less time and presented higher percentages of oxidation and lower percentages of cellular activity than those of *M. deliciosa*. On the other hand, Carvajal-Guillén[11] found that mature leaves of *M. deliciosa* presented greater leaf longevity, less yellowing and absence of mechanical damage, due to the higher content of carbohydrates, proteins and lipids in the cell walls, which is associated with greater rigidity and persistence.

The contamination of explants depends on the biological defenses of the species and the content of endophytic microorganisms. Pérez *et al.*[12] report that the population density of endophytic bacteria is highly variable in hemiepiphytic plants (*M. deliciosa* and *M. acuminata*) and regularly increases in adult plants under warm climate conditions, in habitats with high diversity and low altitude.

The first disinfection protocol was less aggressive for tissues without vacuum pump pretreatment, so only a 2.5% sodium hypochlorite immersion was performed (**Table 4**), this differs from the results of Donini *et al.*[13], who found 80% of *M. deliciosa* tissues with 1.5% active chlorine disinfection.

The medium with the PPM preservative at 1 ml/L presented the lowest levels of oxidation. In this regard, Rihan *et al.*[14] determined that PPM at 1 mL/L is an agent that not only inhibits microbial growth, but also improves the development and quality of shoots *in vitro*. In contrast, moderate use of PVP is necessary to improve survival rates in

sugarcane leaf explants; Shimelis[15] recommends applying 0.2 g/L for *in vitro* micropropagation of this crop.

Aseptic establishment of leaf explants was favorable for *M. deliciosa* as opposed to *M. acuminata* explants; however, dark conditions were ideal for inducing morphogenic responses (bending and elongation) in both species, behavior similar to that found in vivo.

Table 4. Percentages of survival, contamination, cell activity, oxidation, callus formation and number of new shoots per treatment of stem discs in *M. acuminata* K. at 35 days after sowing (Average of at least 15 replicates)

Treatment	Sur (%)	Cont (%)	A.C. (%)	Oxi (%)	Callus (%)	NBT
1. P1 + MS + BAP (1 mg/L) + AIA (0.5 mg/L) + ANA (0.1 mg/L)	84.91	39.68	65.87	58.49	65.87	2
2. P1 + MS + BAP (2 mg/L) + AIA (1 mg/L) + ANA (0.5 mg/L)	68.88	42.22	81.11	73.44	75.55	0
3. P2 + MS + BAP (1 mg/L) + AIA (0.5 mg/L) + ANA (0.1 mg/L)	87.5	7.87	95.23	77.97	65.87	7
4. P2 + MS + BAP (2 mg/L) + AIA (1 mg/L) + ANA (0.5 mg/L)	69.83	4.76	78.30	82.30	34.52	2

P1 = 3% Antibenzyl Soap + 70% Ethanol + 1.25% NaClO + Antioxidant sol., P2 = Tween-20 + 20% Ethanol + 2.08% NaClO + Benomyl solution + cysteine, Sur = survival, Cont = contamination, A.C.= cell activity, Oxi = oxidation, Callus = callus formation, NBT = number of new shoots per treatment.

4.2 Induction of organogenesis in *M. acuminata*

Cellular activity in the stem discs consisted of turgor, elongation and friable callus formation. The organogenic capacity (caulogenesis) was possibly associated with the phenological age and physiological state of the selected shoots, since these came from young plants in pre-flowering stage (<1 m tall), which is consistent with the results of the work of Valderrama-Alfaro *et al.*[16], who found that plasticity and early explant age enhances cell division and influences greater shoot formation.

The hormone balance was different in each explant, as it depended on the original shoot and the determined section (apical, middle, basal). Chesha Desai *et al.*[17] state that in Araceae, explants from vegetative parts contain different endogenous levels of growth regulators, depending on their location in the plant, which explains the different *in vitro* responses, even in the same treatment.

The exogenous concentration of growth regulators that favored shoot formation from stem discs was: BAP (1 mg/L) + AIA (0.5 mg/L) + ANA (0.1 mg/L). The above agrees with the findings of Blanco and Valverde[18], Valderrama-Alfaro *et al.*[16] and Murillo-Gómez *et al.*[19], who recommend the use of BAP at 0.8 and 1 mg/L to induce the best shoot multiplication rates in *Philodendron corcovadense* and *Anthurium* species**,** respectively.

Maintaining stem discs in dark conditions for up to 14 days favored shoot induction in treatments 1, 3 and 4, a result similar to that obtained by Wang *et al.*[20] working on strawberry crop (95%), after 14 days in dark conditions.

The formation of friable callus was present in all explants of the apical section of treatments 1 and 2 and in most of treatment 4. In treatment 3 all explants of the basal (100%) middle (83.33%) and apical (14.29%) section presented this response. These results were similar to those found by Valle-Sandoval *et al.*[7], who in chrysanthemum obtained the lowest percentage of shoot regeneration in the apical zone, and higher in the middle zone of the 1 mm thick stem disk.

Differences were observed between friable callus originating from the central and peripheral part of the stem disc (**Figure 2A**). The induced shoots showed characteristics of axillary origin 45 days after sowing (**Figures 2B** and **2C**) and of adventitious origin, as parenchyma cells with high meristematic activity were observed forming part of the lateral shoot (**Figure 2D**).

5. Conclusions

Aseptic establishment of *M. acuminata* Koch and *M. deliciosa* Liebm. was achieved in this work. The best disinfection protocol for foliar explants was a single dip in 2.5% NaClO, in which all ex-

plants of treatment 8 (*M. deliciosa* + MS (1 mg/L BAP and 0.2 mg/L 2,4-D) +1 mL/L PPM) survived.

The most efficient protocol for disinfection of *M. acuminata* shoots for shoot induction was: Tween-20 + 20 % ethanol + 2.08 % NaClO + benomyl solution + cysteine, being treatment 3 (sown in MS medium with 1 mg/L BAP, 0.5 mg/L AIA and 0.1 mg/L ANA) the one that obtained the explants with the highest sprouting.

In the case of organogenesis induction from stem discs, it was possible to identify conditions and hormonal treatments that favored shoot induction; however, the origin of the shoots remains to be verified and efficient protocols for the induction of adventitious shoots with cell totipotency, which are ideal for *in vitro* propagation and later for *ex situ* conservation, still need to be developed.

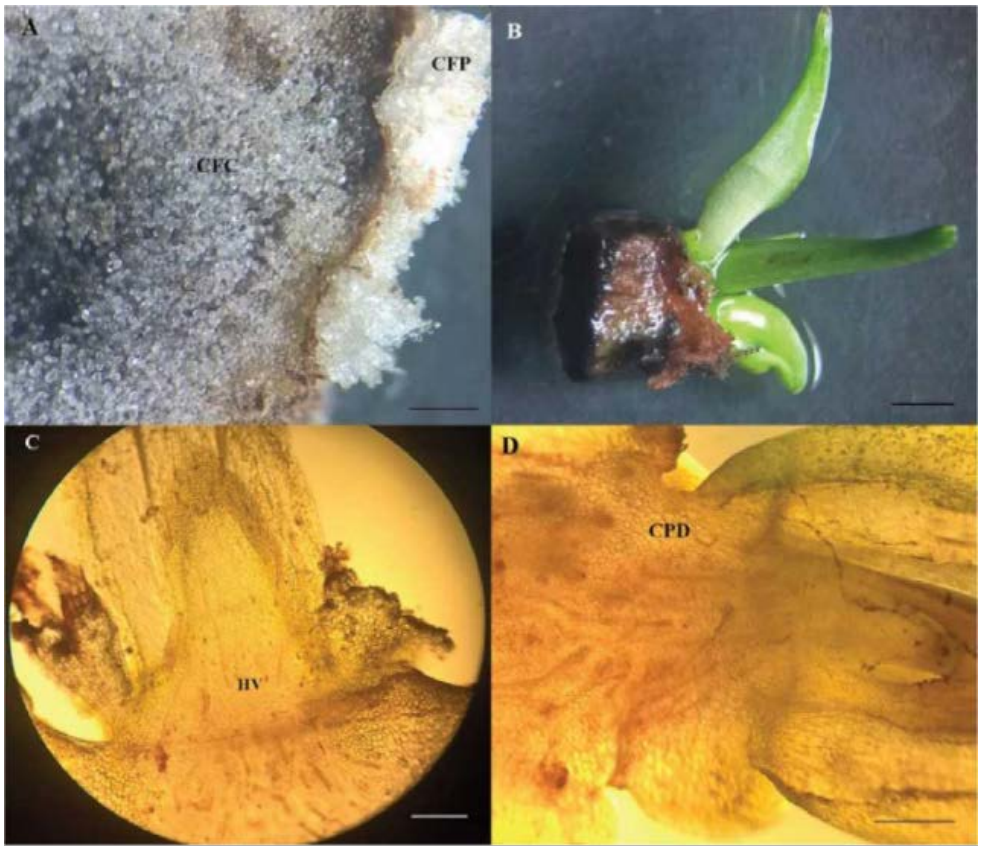

Figure 2. Stem discs of explants from treatment 3 to 35 days old.
A. Central friable callus (CFC) and peripheral friable callus (PFC) in an explant, bar = 1 mm. **B**) Shoots induced 45 days after sowing, bar = 2 mm. **C**) Histological section of main shoot, bar = 2 mm. **D**) Histological section of side shoot, bar = 2.5 mm. HV = vascular bundles CPD = stem disc parenchyma cells.

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Conflict of interest

The authors declared no conflict of interest.

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