La Granja:
Revista de
Ciencias de la Vida

pISSN:1390-3799; eISSN:1390-8596

http://doi.org/10.17163/lgr.n31.2020.05

Scientific paper/ Artículo científico

BIOTECHNOLOGY

Propagation in vitro of qusihuar (Buddleja incana Ruíz & Pav)

Propagación in vitro de quishuar (Buddleja incana Ruíz & Pav)

Paola Jiménez Enriquez[1], Paulo Barrera Aguilar[2], Laura Huachi Espín*[3], Antonio Vera Zambrano[4] and Carlos Caicedo Vargas[5]

[1] *Biotechnology Engineer, Universidad Politécnica Salesiana. Sede El Girón, Av. 12 de Octubre y Wilson, Quito, Ecuador.*
[2] *Master in Forestry Sciences, National Institute of Agricultural Research. Experimental station at Amazonía. Sacha- San Carlos 3 Km from the entrance to Parker, Joya de los Sachas parish, Orellana, Ecuador.*
[3] *Master in Environmental Management. Specialist in Nutrition and plant fertilization. Universidad Politécnica Salesiana, Campus El Girón. Av. 12 de Octubre and Wilson, Pichincha- Ecuador.*
[4] *Master in Agroforestry, National Institute of Agricultural Research. Experimental station at Amazonía. Sacha- San Carlos 3 Km from the entrance to Parker, Joya de los Sachas parish, Orellana. Ecuador.*
[5] *Agronomy Engineer, National Institute of Agricultural Research. Experimental station at Amazonía. Sacha- San Carlos 3 Km from the entrance to Parker, Joya de los Sachas parish, Orellana, Ecuador.*

*Corresponding author: lhuachi@ups.edu.ec

Article received on may 8th, 2019. Accepted, after review, on february 4th, 2020. Published on March 1st, 2020.

Resumen

Quishuar es una especie forestal apreciada por sus usos. La explotación intensiva y la oferta insatisfecha de plantas han sido limitantes para cumplir con programas de reforestación. La micropropagación es una técnica que ayudaría a erradicar este problema ya que el propósito es producir mayor cantidad de plantas en menor tiempo. El objetivo de esta investigación fue desarrollar protocolos para la desinfección, establecimiento y multiplicación *in vitro*, para el efecto se realizaron ensayos partiendo de semillas y brotes de plantas. La primera fase se realizó con semillas, utilizando kilol y benomil, junto con NaOCl al 3 % (10 min). Los resultados indican que el porcentaje de germinación fue de 100 % en MS (Murashige y Skoog medium) y el porcentaje de contaminación y oxidación de 0 %. Por otro lado, los brotes sometidos a fungicidas con adición de antioxidantes y NaOCl al 1 % (10 min) no presentaron contaminación ni oxidación. El porcentaje de brotación fue de 100 % en WPM (Woody Plant Medium). En la segunda fase en medio MS sin adición de hormonas se observó una mayor longitud de brote (1.95 cm), número de nudos (1.94 nudos) e índice de multiplicación (2.47). Basándose en los resultados, se sugiere que los protocolos son efectivos para la propagación *in vitro*.

Palabras clave: contaminación, in vitro, micropropagación, oxidación, Quishuar

Abstract

Quishuar is a forest species which is well known for its uses. Intensive farming and unsatisfied plant supply have been limited to meet reforestation programs. Micropropagation is a technique that would help eradicate this problem as the purpose is to produce more plants in less time. The objective of this research was to develop protocols for disinfection, establishment, and multiplication *in vitro*; for this reason, tests from seeds and plants sprouts were carried out; the first phase with seeds, using kilol and benomil, together with NaOCl at 3 % (10 min). The results indicate that the germination percentage was 100 % in MS (Murashige y Skoog medium) and the contamination, oxidation percentage was 0 %. On the other hand, plants sprouts exposed to fungicides, antioxidants and NaOCl at 1 % (10 min), did not have contamination or oxidation. The sprouting percentage was 100 % in WPM (Woody Plant Medium). In the second phase in MS medium without the addition of hormones, plant sprouts (1.95 cm), knots number (1.94 knots) and multiplication rate (2.67) were observed. Based on the results, it is suggested that protocols are effective for *in vitro* propagation.

Keywords: contamination, in vitro, micropropagation, oxidation, Quishuar

Suggested citation: Jiménez, P., Barrera, P., Huachi, L., Vera, A. and Caicedo, C. (2020). Propagation *in vitro* of qusihuar (*Buddleja incana Ruíz & Pav*). La Granja: Revista de Ciencias de la Vida. Vol. 31(1):74-84. http://doi.org/10.17163/lgr.n31.2020.05.

Orcid IDs:
Paola Jiménez Enriquez: https://orcid.org/0000-0001-7026-8537
Paulo Barrera Aguilar: https://orcid.org/0000-0003-1465-1114
Laura Huachi Espín: https://orcid.org/0000-0002-5367-3714
Antonio Vera Zambrano: https://orcid.org/0000-0002-9136-8825
Carlos Caicedo Vargas: https://orcid.org/0000-0002-2777-5282

1 Introducion

Ecuador is considered a country with a forest vocation and yet this sector contributes little to the nation's economy. There are about 7 million hectares of forests with a forest management potential lower than 10 %, but which are economically eligible to be subjected to sustainable forest management (Sánchez, 2012).

Forest genetic resources FGR are being lost at an alarming rate due to indiscriminate use and lack of incentives for the preservation and its sustainable use. The knowledge of FGR is still precarious and insufficient, studies and institutions carrying out activities for their protection are scarce, and the current availability of specific information on the situation, trends and recovery of FGR is deficient (Ministerio del Ambiente-MAE, 2005).

Thus, Quishuar (*Buddleja incana*) is a tree of the Scropulariaceae family with 15 m of height approximately, with flowers grouped on small heads and fruits of 5-6 mm of length. This tree is a forest species native to Ecuador, located in the provinces of Chimborazo, Pichincha, Tungurahua, Azuay, Loja and Imbabura (Grijalva et al., 2012). Wood is used for the making of plows, rudders, hoe capes, poles, stakes, crafts, house building and corrals. In addition, the infusion of leaves is used for medicinal purposes such as antirheumatic, healing, antibacterial and antifungal, as well as to stimulate the proliferation of the endometrium and regenerator of the skin; in mice it has been shown to inhibit cyclooxygenase (COX_2) (Gómez, 2006).

The propagation through the conventional methods established for Quishuar has several problems; the multiplication in nursery requires 5 months, resulting in higher production costs and less plant production per year (Gárate, 2010). The amount of vegetal material available is insufficient for afforestation, reforestation in degraded areas of wastelands and watersheds, and one of the strategies for overcoming conventional propagation difficulties is *in vitro* cultivation of vegetable tissues (Delgado et al., 2008). *In vitro* culture is a technique of plant production under totally aseptic conditions; it is based on cell potency, i.e., the ability of a plant cell to form a complete plant under certain conditions. Thus, the rapid and massive propagation of plants identical to the original is achieved from any part of the plant, whether pieces of tissues, meristhematic apices or even isolated cells (Reyes and y Hewstone, 1994).

According to Vallejo (1988), the spread of this species at the laboratory level presents some problems such as the length of shoots 8.6 mm, low percentage of number of knots per plant (1.5 knots), but there are no reported data regarding pollution. However, Cárdenas (2011), states that pollution is the main limiting in the *in vitro* development of this forest species, since the survival rate does not exceed 54 %, mainly due to fungal and bacterial conditions.

Due to the difficulties of conventional propagation and the results obtained in the laboratory by Cárdenas (2011) and Vallejo (1988), it has been proposed to establish a method for the disinfection, establishment and multiplication of Quishuar through the application of *in vitro*, culture techniques, because it is essential to generate strategies that allow the propagation and conservation of this species.

The aim of this research was to develop a protocol for the *in vitro* propagation of Quishuar (*Buddleja incana*).

2 Methodology

This study was conducted in the plant tissue culture laboratory of the National Department of Biotechnology at INIAP (National Agricultural Research Institute), Cutuglahua, Mejía parish, Pichincha Province and in the Kichwa communities located in the mountain range.

3 Vegetal Material and Explant Preparation

Seeds and buds (axillary and apical) of Quishuar plants in the Kichwa San Juan community, Chimborazo, were used. Prior to the research the plants were moved to the greenhouse of the National Department of Biotechnology, fumigated every 8 days with an application of Carbendazim 1 $ml\ l^{-1}$ and Skul Fe (Thiodicarb) 1 $ml\ l^{-1}$ to prevent contamination, the

plants were fertilized every 15 days with Stimufol 1 $g\ l^{-1}$ and irrigation was performed twice a week with 200 $ml\ l^{-1}$ of drinking water per plant (Laboratorio de Cultivo de Tejidos (LCT-INIAP), 2014).

4 Propagation Phases *in vitro*

Disinfection and establishment phases. The collected seeds were placed in a container with a solution of water and detergent, then were rinsed with running water until all residues were removed. Once washed, they were immersed in a 1 % Povidone solution (Iodine-povidone) for 60 minutes, then were transferred to a Benomyl solution (Benzimidazole) 0,10 $g\ l^{-1}$ adding 30 drops of Kilol (Citrus paradisi). The seeds were taken to a laminar flow chamber, where 2 rinses with distilled water were made and immediately were immersed in a solution of Sodium Hypochlorite (NaOCl) 3 % for 10 minutes, subsequently 5 rinses were performed with distilled sterile water. The seeds were sown in the culture medium MS (Murashige and Skoog medium) and WPM (Woody Medium Plant), incubated 18 $°C$ for 30 days.

On the other hand, buds (axillary and apical) were also washed with a solution of water with detergent and running water to remove all detergent residues. Subsequently, these were disinfected in a Povidone solution (Iodine−povidone) 1 % for 60 minutes, and then were transferred to a fungicide solution (Phyton 0,5 $ml\ l^{-1}$ + Carbendazim 0,5 $ml\ l^{-1}$), antioxidants (Ascorbic acid + Citric acid 0,1 $g\ l^{-1}$),30 drops of Kilol *Citrus paradisi* and activated charcoal 0,5 $g\ l^{-1}$ for 20 minutes. Buds were placed in a laminar flow chamber with two solutions of Sodium Hypochlorite (NaOCl), the first 0,5 % for 60 minutes and the second 1 % NaOCl with the addition of 3 drops of Tween 20 for 10 minutes, with 5 rinses of sterile distilled water. Buds were planted in two MS and WPM culture media and incubated at 21 $°C$ for 30 days (Laboratorio de Cultivo de Tejidos (LCT-INIAP), 2014).

Multiplication phase. Explants of 2-3 cm of length were cut from the plants obtained from seeds, which were transferred to different culture media, MS and WPM without the addition of hormones and with the addition of 0,5 $g\ l^{-1}$ and 0,1 $g\ l^{-1}$ of BAP (Benzyl adenine) and gibberellic acid AG_3 (Hernández et al., 1999). 10 observations were made, with evaluations at 30 and 45 days.
Explants of 1-3 cm of length were cut from plants obtained from shoots, then were transferred to culture media MS and WPM without the addition of hormones and with the addition of 0.5 $g\ l^{-1}$ and 0,1 $g\ l^{-1}$ of BAP (Benzyl adenine) and gibberellic acid AG_3 (Hernández et al., 1999).

Incubation and photoperiod of vitroplants. During the multiplication phase, 5 explants were placed per bottle, sealed with parafilm paper to avoid contamination, kept in the growing room with light intensity of 2000 luxe at a temperature of 18 $°C$ and 21 % humidity. Plants were evaluated every 30 days.

5 Experimental Design and Statistical Analysis

A split plot design was used for the analysis, for the first phase the plot design was (3×2) with six treatments and a second phase with a plot design (2×2×3) with 12 treatments. 10 observations were used in each stage.

PFor the data, a variance analysis (ADEVA) and mean separation were performed at 95 % de probabilidad. probability. To establish statistical differences between treatments, the Tukey Multiple Range Test (p <0.05). was performed. The data that had a value of 0 were transformed with the formula $\sqrt{x+1}$, in order to decrease the variation coefficient and adjust the data to the normal distribution (Vinueza, 2013).

6 Results and Discussion

6.1 Disinfection and Seed Setting Phase

In several researches conducted by the Statistics Division of the Nutrition Institute of Central America and Panama (INCAP) it is stated that laboratory studies using the following control measures: homogeneous experimental material, external factor control, the allocation of few experimental units to treatments and the refinement of the techniques applied in the research, result in the continuation of

Table 1. Variables evaluated in the disinfection stage and *in vitro* establishment of *B. incana* seeds.

Treatment	Protocol	Medium	Contamination percentage	Oxidation percentage	Germination percentage	Bud length (cm) 30 days	Budlength (cm) 45 days
T1×10REP	Povidone solution 1 % 60′ Fungicides (Phyton 1 *ml* l^{-1} + distilled water + Carbendazim 1 *ml* l^{-1} distilled water) + 60 NaOCl 1 % Tween 20 10′.	MS	0	0	100	1.22±0.03	1.37±0.05
T2×10REP	Povidone solution 1 % 60′ Fungicides (Phyton 1 *ml* l^{-1} + distilled water + Carbendazim 1 *ml* l^{-1} distilled water) + 60 NaOCl 1 % Tween 20 10′.	WPM	20	0	100	1.22±0.03	1.44±0.05
T3×10REP	Povidone solution 1 % 60′ + Kilol 30 drops l^{-1} + distilled water 30′ + Benomyl 1 *g* l^{-1} + NaOCl 3 % 10′.	MS	0	0	100	1.33±0.03	1.47±0.05
T4×10REP	Povidone solution 1 % 60′ + Kilol 30 drops l^{-1} + distilled water 30′ + Benomyl 1 *g* l^{-1} + NaOCl 3 % 10′.	WPM	0	0	100	1.27±0.03	1.40±0.05
T5×10REP	Povidone soap 1 % 30′ + Fungicides (Benomyl 1 *g* l^{-1} water + Phyton 0.5 *ml* l^{-1} distilled water) + 30′ + NaOCl 1 % 8′.	MS	20	0	100	1.07±0.03	1.14±0.05
T6×10REP	Povidone soap 1 % 30′ + Fungicides (Benomyl 1 *g* l^{-1} water + Phyton 0.5 *ml* l^{-1} distilled water) + 30′ + NaOCl 1 % 8′.	WPM	10	0	100	1.15±0.03	1.35±0.05

the same experimental error or that it is feasible to reduce the error factor and keep it under control. Research of the experimental error used as an example of processes clearly indicates that the investigation of the error cannot be just a mere part of a complete process, but it should be considered as one more activity within the framework of general techniques to be applied during any kind of investigation (Guzmán, 1975).

The variables evaluated at this stage were percentage of contamination, oxidation, germination and bud length.

Contamination by fungi and/or bacteria was observed in T2, T5 and T6 treatments with a percentage of 100 %. Treatments T1, T3 and T4 resulted in explants without contamination within 30 days of evaluation. The oxidation rate for treatments T1, T2, T3, T4, T5 and T6 was 0 %.

Treatments T1, T3 and T4 reported 100 % seed germination at 30 days, and this percentage was maintained for up to 45 days. In T1 and T3 treatments the MS culture medium was used as well as the WPM culture medium for T4 treatment. For the variable bud length, Tukey 5 % test was performed to establish significance ranges, for T3 (MS) increased growth was observed with an average of 1.33 ± 0.03 cm. . For T4 (WPM) a growth of 1,27 ± 0.03 cm and for T1 (MS) a growth of 1.22 ± 0.03 cm at 30 days of evaluation. At 45 days, growth was evident for T3 (MS) with an average of 1.47 ± 0.05 cm. For T4 (WPM) a growth of 1.40 ± 0.05cm , and for T1 (MS) a growth of 1.37 ± 0.05 cm.

The results obtained at this stage agree with those mentioned by the authors. In Billard et al. (2014), NaOCl 1 % 10′ 10′ was used in *Oncidium bifolum* seeds reporting 0 % of contamination and oxidation; it further mentions that when using MS culture medium, the germination rate was 100 %. However, the bud length was smaller compared to the T3 and T4 treatment, this may be due to González (2010), in his study with *Musa paradisiaca* mentions that the use of fungicides/bactericides (Phyton) at doses higher than 0.5 *ml* l^{-1} could slow the growth of explants, this is because when the product is rapidly absorbed by the leaves and roots, it causes plant toxicity, reducing branching and causing plant deterioration. These results would be corroborated by those obtained from T1 treatment.

On the other hand, Soto et al. (2010), used NaOCl 3 % 10′ in *Cedrela salvadorensis* seeds, reporting 0 % of contamination and oxidation; NaOCl is

recommended as a surface disinfectant as its mechanism of action allows damage to the cell membrane of bacteria, causing the lysis of the microorganism. In addition, they mentioned that seed germination was 65 % for the WPM culture medium and 100 % for the MS culture medium. The MS culture medium contains a high concentration of salts while the WPM culture medium contains reduction of the total mineral salts. Bud length in T3 was longer compared to T1 and T4 treatment.

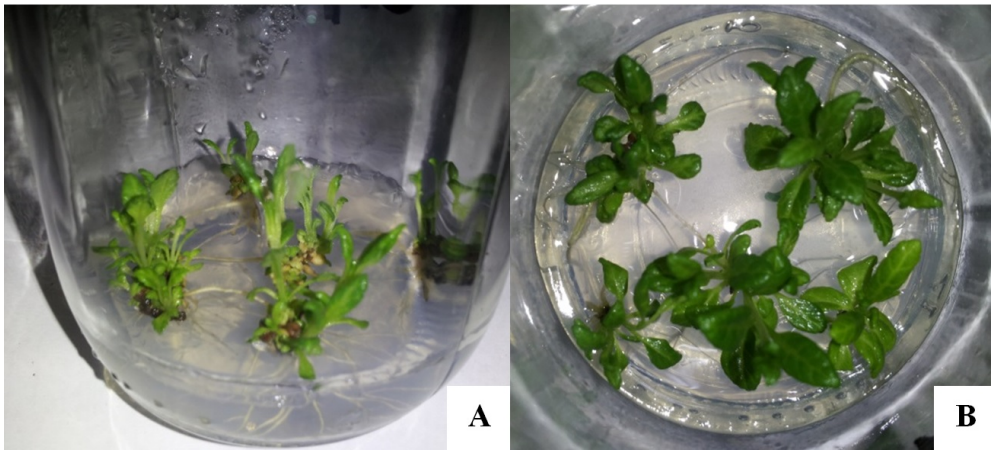

Figure 1. A and B) Treatment T3. Plants in the MS culture medium at 30 days of evaluation.

7 Dissinfection and Settins of Buds

The variables evaluated in this phase were percentage of contamination, oxidation, sprouting, number of knots and bud height.

Contamination by fungi and/or bacteria was observed in T1, T2, T3 and T4 treatments with a percentage of 70, 50, 40 and 80 %,respectively. For T5 and T6 treatments, the results showed explants free of contamination at 30 days of evaluation. The oxidation rate for T1, T2, T3, T4, and T6 treatments was 0 %, while for T5 was 20 %. The percentage of sprouting for treatments T5 (MS) and T6 (WPM) was 80 and 100 %, respectively. Tukey 5 % test was used for the variable number of knots and bud height to differentiate significance ranges, obtaining an average 1.61 $\pm$ 0.1 knots and one mean in bud length for the MS culture medium of 1.45 $\pm$ 0.08 cm, and 1.55 $\pm$ 0.08 cm. for the WPM culture medium. Sodium hypochlorite was effective for disinfection of buds during *in vitro* establishment of *C. spinosa*. In this phase of *in vitro* different compounds can be used for the disinfection of plant material, and solutions of sodium hypochlorite and alcohol at different percentages are the most common products (Azofeifa, 2009).

The results obtained in this phase are higher compared to those mentioned by Cárdenas (2011), who used buds of Quishuar from mother plants that were two years old, and stated that the disinfection process was with NaOCl 2,5 % for 10 min, obtaining 70 % survival. This research used 1-year-old buds and the modified LCT-INIAP disinfection protocol, obtaining 100 % survival. Oxidation in the T5 treatment may have been due to environmental factors such as light intensity, cuts, senescence, heavy metals and lesions that can trigger oxidative stress (Luna et al., 2003). Parada (2009), mentions in his study with *Prunus persica* that a large number of knots (3-4) were obtained when using the WPM culture medium and the length was 13.7 mm, this because the high concentration of salts in the MS culture medium can cause a delay in sprouting and can be toxic to tissues, as is the case of *Vaccinium corymbosum*. This species responded better in the WPM culture medium as it contains reduction of the total amount of mineral salts (Sedlak and Paprstein, 2009). The best results were observed in T6 treatment (WPM).

Table 2. Variables evaluated at the disinfection stage and *in vitro* establishment of *B. incana* buds

Treatment	Protocol	Medium	Contamination Percentage	Oxidation Percentage	Bud Percentage	Percentage of knots (30 days)	Bud length (30 days)
T1×10REP	Povidone Solution 1 % 20′ + Fungicides (Benomyl 1 $g\ l^{-1}$ + Phyton 1 $ml\ l^{-1}$ + Rinfapicina 0.5 $ml\ l^{-1}$) 60′ + Alcohol 70 % 1′ + NaOCl 2 % 15′.	MS	70	0	30	1.22±0.1	1.17±0.08
T2×10REP	Povidone Solution 1 % 20′ + Fungicides (Benomyl 1 $g\ l^{-1}$ + Phyton 1 $ml\ l^{-1}$ + Rinfapicina 0.5 $ml\ l^{-1}$) 60′ + Alcohol 70 % 1′ + NaOCl 2 % 15′.	WPM	50	0	50	1.30±0.1	1.27±0.08
T3×10REP	Povidone Solution 1 % + Kilol 20 drops l^{-1} + distilled water 30′ + Benomyl 1 $g\ l^{-1}$ 90′ + Alcohol 70 % 30″ + NaOCl 1 % 15′ + Activated carbon (0,5 $g\ l^{-1}$).	MS	40	0	60	1.41±0.1	1.33±0.08
T4×10REP	Povidone Solution 1 % + Kilol 20 drops l^{-1} + distilled water 30′ + Benomyl 1 $g\ l^{-1}$ 90′ + Alcohol 70 % 30″ + NaOCl 1 % 15′ + Activated carbon (0,5 $g\ l^{-1}$).	WPM	80	0	20	1.11±0.1	1,11±0.08
T5×10REP	Povidone solution 1 % 60′ + Activated carbon 0.5 $g\ l^{-1}$ + Fungicides (Phyton 0.5 $ml\ l^{-1}$ + Carbendazim 0.5 $ml\ l^{-1}$) + Kilol 30 drops/ 100 ml + Ascorbic acid 0.1$g\ l^{-1}$ 20′ + NaOCl 0.5 % 60′ + NaOCl 1 % 10′ + Tween 20	MS	0	20	80	1.55±0.1	1.45±0.08
T6×10REP	Povidone solution 1 % 60′ + Activated carbon 0.5 $g\ l^{-1}$ + Fungicides (Phyton 0.5 $ml\ l^{-1}$ + Carbendazim 0.5 $ml\ l^{-1}$) + Kilol 30 drops l^{-1} + Ascorbic acid 0.1$g\ l^{-1}$ 20′ + NaOCl 0.5 % 60′ + NaOCl 1 % 10′ + Tween 20	WPM	0	0	100	1.67±0.1	1.55±0.08

8 Multiplication Phase

The variables evaluated in this phase were bud height, number of knots, multiplication rate and root length. Plants obtained from the seed phase of the T3 treatment were used. The MS culture medium without the addition of BAP (6-Bencilaminopurin) showed the best result, obtaining a bud height of 1.95 ± 0.05 cm and number of knots 1.94 ± 0.04, followed by MS without addition of AG_3 ((Gibberellic acid) with an average of 1.78 ± 0.05 cm and number of knots of 1.79 ± 0.04 after 30 days of evaluation. These results are higher than those mentioned by Cárdenas (2011), in which the bud height obtained was 0.4 cm and the number of knots 1.25, using MS without the addition of hormones. In the study with *C. espinosa*, the concentration of BAP (6-Bencilaminopurin) did not significantly influence the number of initial explants regarding the control treatment. However, the addition of 0.25 and 0.50 $mg\ l^{-1}$ significantly favored the number of buds obtained from an initial explant. The lowest concentration of BAP (6-Bencilaminopurin) 0.25 $mg\ l^{-1}$ was selected as the best treatment for this phase of the process, with 96.6 % sprouting and in 30 days of cultivation an established vigorous explant was achieved, with an average of 2.07 buds of 6.71 cm long (Núñez et al., 2017).

The multiplication rate is the arithmetic mean of the number of buds generated at 30 days in each treatment. It was 2.60 for the culture medium MS without the addition of BAP (6-Bencilaminopurin), and 2.38 for MS without the addition of AG_3 (Gibberellic acid). The MS culture medium without the addition of hormones guaranteed a multiplication rate of 2.60.

García et al. (2018), mentions that culture medium supplemented with hormones such as BAP (6-

Bencilaminopurin) promoted proliferation of axillary buds, but also caused physiological effects (abundant callus growth at the base of seedlings being this negative) as evidenced in treatments with the addition of BAP (6-Bencilaminopurin) at 0.1 $g\ l^{-1}$. Different authors refer to the positive effect of 6-BAP and ANA (1-naftalenactic acid) for *in vitro* multiplication of other tree legume species, such is the case of Rahman et al. (1993), who during the *in vitro* establishment of nodal segments of *C. pulcherrima*, reported a multiplication coefficient of 5.8 new buds/explant with a MS culture medium combined with 1.0 $mg\ l^{-1}$ of BAP (6-Benaminocilpurin) and + 1,0 $mg\ l^{-1}$ of ANA (1-l-naftalen acetic acid).

Agramonte et al. (2001), when evaluating the effect of different doses of 6- BAP (0; 0.35; 0.50 and 1.0 $mg\ l^{-1}$) on *in vitro* multiplication of *Eucaliptus grandis*, observed a tendency to increase the values of the variable number of buds per explant, and a reduction in their length with increased concentration, thus demonstrating that relatively high doses induce a high axillary tilling and reduce the size of the bud, resulting in an affectation of the multiplication coefficient.

The highest average of root length was for the MS culture medium without the addition of hormones with an average 2.25 $\pm$ 0.11 cm. Saucedo et al. (2009), in the research with *Xanthosoma sagittifolium*, mentions that the use of cultured medium without the addition of BAP (6-Bencilaminopurin) in the rooting phase developed longer roots, obtaining an average of 4.5 cm. The use of BAP (6-Bencilaminopurin) caused symptoms of hypehydricity in plants.

In general, Murashige and Skoog (1962) suggested that the concentration management of mineral salts is widely recommended to stimulate rooting, the formation of buds, leaves and the length of plants *in vitro*, while Piqueras (2000) observed that the concentration of mineral salts can affect the morphology of micropropagated plants through changes in osmotic pressure, mainly affecting the development of *in vitro* roots.

Jiménez et al. (2016) in their study with *Dianthus caryophyllus*, in assessing the effect of the concentration of inorganic salts of the culture medium in relation to the length of the plant did not observe differences between treatments, except with the witness, thus maybe it had the nutrients needed for its growth. However, a reduction in the growth was seen with the decrease in the concentration of mineral salts, being lower in the treatment where 25% was used. These results show that the decrease of the mineral content in the culture media favored the growth retardation, due to alterations occurring in the cellular metabolism.

Figure 2. A) Multiplication of vitroplants (MS) in a culture medium without any addition of hormones. B) Root length evaluation. C) Callus formation at the base of plants in a culture medium (MS) with the addition of BAP (0,1 $g\ l^{-1}$).

9 Conclusions

The best treatment for the disinfection phase and *in vitro* establishment of *Buddleja incana* seeds was T3 (Povidone solution 1% 60′ + Kilol 30 drops l^{-1} + distilled water 30′ + Benomil 1 $g\ l^{-1}$ + NaOCl 3% 10′), with a contamination rate of 0% and survival rate of 100%. The T3 treatment contained MS culture medium that was effective for the variable bud length, as the highest values were 1.33 ± 0.03 cm at 30 days and 1.47 ± 0.03 cm at 45 days. According to Jiménez et al. (2001), it is important to take into account the area of the tissue used to start *in vitro*, cultivation, since it has a great influence on the efficiency of disinfection.

The *in vitro* establishment phase of *Buddleja incana* buds, showed that the best treatment was T6 (Phyton 0.5 $ml\ l^{-1}$ + Carbendazim 0.5 $ml\ l^{-1}$) + Kilol 30 drops l^{-1} + Ascorbic acid $0.1g\ l^{-1}$ 20′ + NaOCl 0.5 % 60′ + NaOCl 1 % 10′ + Tween 20) with 0% contamination. The bud percentage in this treatment was 100%. The number and length of buds had an average of 1.67 ± 0.1 buds/explant and 1.55 ± 0.08 cm of length. In the work carried out by Gutiérrez (2002), in *Alnus acuminata* from nod segments and leaf, a disinfection protocol was used with very similar concentrations of NaClO and times proposed in Marulanda and Isaza (2004). The results indicated that disinfection treatment with NaClO at 1% resulted in less oxidation in the leaves than in the nodal segments.

In *in vitro* multiplication essays of *Buddleja incana*, the MS culture medium presented the best results in treatments without the addition of the hormones BAP and AG_3. The bud length had an average of 1.95 ± 0.05 cm, the number of knots had an average of 1.94 ± 0.04 knots/explant and multiplication rate of 2.60. The results obtained in the multiplication phase showed that no rooting promoter was required since the root length had an average of 2.25 ± 0.08 cm.

This research represents an initial phase of establishment and *in vitro* multiplication, where the results indicate a probability of success in the micropropagation process of this species; however, feasibility studies in the field still need to be performed.

References

Agramonte, D., Delgado, L., Trocones, A., Pérez, M., Ramirez, D., and Gutiérrez, O. (2001). Micropropagación del eucalyptus grandis (hill ex maiden) a partir de segmentos nodales. *Biotecnología vegetal*, 1(2):109–114. Online: https://bit.ly/39X58x4.

Azofeifa, A. (2009). Problemas de oxidación y oscurecimiento de explantes in vitro. *Agronomía mesoamericana*, 20(1):153–175. Online: https://bit.ly/2uozSrY.

Billard, C. E., Dalzotto, C. A., and Lallana, V. H. (2014). Desinfección y siembra asimbiótica de semillas de dos especies y una variedad de orquideas del género oncidium. *Revista Polibotanica*, (38):145–157. Online: https://bit.ly/32emkf9.

Cárdenas, M. (2011). Determinación del protocolo de establecimiento y multiplicación in vitro de quishuar (buddleja incana) a partir de yemas axilares de plantas madre, como una herramienta para la preservación de esta especie dentro del distrito metropolitano de quito.

Delgado, M., Hechenleitner, P., and Thiers, O. (2008). Propagación vegetativa de taique (desfontainia spinosa) y tepa (laureliopsis philippana) con fines ornamentales. *Revista Bosque*, 29(2):120–126. Online: https://bit.ly/2VaTpa7.

Gárate, M. (2010). Técnicas de propagación por estacas.

García, L., Moreno, L., Pérez, M., La O, M., Padrón, Y., Rodríguez, Y., and Rivero, L. (2018). Protocolo para la micropropagación de anthurium cubense engler a partir de semilla botánica. *Biotecnología Vegetal*, 18(3):161–166. Online: https://bit.ly/2vZvcZN.

Gómez, M. (2006). Evaluación de alternativas silvopastoriles utilizando yagual (polylepis racemosa), quishuar (buddleja incana), colle (buddleja coriacea) en la microcuenca del río chimborazo.

González, H. (2010). Evaluación de la eficacia in vitro de productos naturales y químicos en el control de especies fúngicas que afectan al cultivo de musa paradisiaca. Technical report, Informe técnico (1).

Grijalva, J., Ramos, R., Checa, X., Anti, A., Barrera, P., and Telenchano, A. (2012). Gestión sostenible de microcuencas en los andes. instituto nacional de investigaciones agropecuarias, programa nacional de forestería, quito. *Revista Informativa INIAP*, (7):11–13. Online: .

Gutiérrez, L. G. (2002). *Embriogénesis somática en Alnus acuminata H.B.K. y estudio de la variación somaclonal mediante marcadores moleculares*. Tesis doctoral.

Guzmán, M. (1975). El error experimental en la investigación científica: Cuantificación de elementos contribuyentes. Technical report, Boletín de la Oficina Sanitaria Panamericana. Online: https://bit.ly/2PgCyir.

Hernández, C., Lopera, M., Mora, B., and Cárdenas, J. (1999). Desarrollo de un protocolo para la propagación masiva de la mora de castilla (rubus glaucus bent) mediante la utilización del cultivo de tejidos vegetales in vitro. *Revista Actualidades Biológicas*, 21(70):3–12.

Jiménez, L., Silva, J., Borges, M., and Fonseca, M. (2016). Conservación in vitro de clavel español (dianthus caryophyllus l) a partir de sales minerales. *Revista Agronomía Mesoamericana*, 27(1):Online: https://bit.ly/32iCgwT.

Jiménez, V. M., Castillo, J., Tavares, E., Guevara, E., and Montiel, M. (2001). Micropropagación de guadua angustifolia kunth a partir de explantes nodales. In *Memorias del Simposio Internacional Guadua. Pereira, Colombia: Universidad Tecnológica de Pereira. Online: https://bit.ly/2SOooY9*.

Laboratorio de Cultivo de Tejidos (LCT-INIAP) (2014). Informe técnico. protocolos de desinfección de especies forestales. Technical Report 1, Informes Iniap.

Luna, C., Sansberro, J., Mroginski, L., and Tarragó, J. (2003). Micropropagation of ilex dumosa from nodal segments in a tissue culture system. *Biocelll*, 27(2):120–135. Online: https://bit.ly/3a0IIeJ.

Marulanda, M. L. and Isaza, L. (2004). Establecimiento in vitro de heliconias con fines de producción masiva. *Scientia et Technica*, (26):193–197. Online: https://bit.ly/2VeUBcC.

Ministerio del Ambiente-MAE (2005). Informe técnico de árboles del ecuador. Technical report, Ministerio del Ambiente, Ecuador.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and biossays with tobacco tissue cultures. *Physiological Plantarum*, 15:473–497. Online: https://bit.ly/2uXxNUh.

Núñez, J., Quiala, E., De Feria, M., Mestanza, S., and Teanga, S. (2017). Propagación in vitro de caesalpinia spinosa (mol.) o. kuntz a partir de yemas axilares de árboles plus seleccionadas. *Revista Biotecnología Vegetal*, 17(2):67–75. Online: https://bit.ly/38RGM87.

Parada, D. (2009). Propagación in vitro del híbrido almendro x durazno h1. *Revista Fitotecnia mexicana*, 32(2):103–109. Online: https://bit.ly/3bXmGLu.

Piqueras, A., y. D. P. . . I. . E. b. A. P. p. .-. (2000). *Morphogenesis in micropro Morphogenesis in micropropagation*, pages 443–462. Online: https://bit.ly/2HMzDtH. Kluwers Academics Publishers.

Rahman, S. M., Hossain, M., Biswas, B. K., Joarder, O. I., and Islam, R. (1993). Micropropagation of caesalpinia pulcherrima through nodal bud culture of mature tree. *Plant Cell, Tissue and Organ Culture*, 32:363–365. Online: https://bit.ly/2Pg4Gm2.

Reyes, H. and y Hewstone, N. (1994). Cultura de tejidos en la agricultura. *Revista Tierra Adentro*, (24):30–33. Online: https://bit.ly/2SQ5HDy.

Sánchez, Y. (2012). Elaboración de tablas de volúmenes y determinación de factores de forma de las especies forestales: Chuncho (cedrelinga catenaeformis), laurel (cordia alliadora), sangre de gallina (otoba sp.), ceibo (ceiba samauma) y canelo (nectandra sp.), en la provincia de orellana.

Saucedo, S., Ramos, L., and Reyes, T. (2009). Efecto de los reguladores de crecimiento para la propagación in vitro de la malanga (xanthosoma sagittifolium (l) schott). *Revista Cienica y Tecnología*, 1(1):17–21. Online: https://bit.ly/37V65VD.

Sedlak, J. and Paprstein, F. (2009). In vitro multiplication of highbush blueberry (vaccinium corymbosum l.) cultivars. In *ISHS Acta Horticulturae*, pages 575–580. Online: https://bit.ly/3c0T2Fi.

Soto, B., Valverde, L., Rojas, A., and Gómez, A. (2010). Establecimiento in vitro de cedrela salvadorensis standl. *Revista Tecnología en marcha*, 23(4):66–73. Online: https://bit.ly/2VemQbC.

Vallejo, S. (1988). Multiplicación a través de cultivo de tejidos vegetales de las especies: quishuar (buddleja incana h.b.k.), cedro (cedrela montana vill) y pumamaqui (oreopanax ecuadorensis seemen).

Vinueza, D. (2013). Evaluación morfológica e histológica de las etapas de formación de la embriogénesis somática en las variedades duke 7 y puebla de aguacate (persea americana mill).