In vitro establishment of nodal segments of Alternanthera porrigens (Moradilla)

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Abstract

This research aimed to find the optimal conditions for the in vitro propagation of Alternanthera porrigens. For disinfection tests and determination of the percentage of viability of the explants, 70% ethanol was used with different concentrations of NaClO (1.0%, 1.5% and 2%) at different exposure times, obtaining the lowest percentage of contamination using 70% ethanol and NaClO 2.0% for 2 and 7 minutes, respectively.

Subsequently, in vitro propagation assays were performed in Murashige and Skoog (MS) culture media supplemented with growth regulators 6-benzylaminopurine (BAP) and 3-indol-acetic acid (AIA) to evaluate the effects on stem elongation, shoot generation (axillary buds) under light conditions for 20 days at 20 ± 3°C.

Concentrations of 0, 0.1, 0.5 mg/l AIA and 0, 0.5 and 1 mg/l BAP were used where the treatment with 0.5mg/l AIA and 1 mg/l BAP was the phytohormone combination that presented optimal results for propagation. Therefore, this work can be presented as a reproducible protocol for in vitro propagation of Alternanthera porrigens.

Keywords: Alternanthera porrigens, shoots, in vitro propagation, growth regulators, nodal segments

Resumen

El objetivo del presente trabajo de investigación fue de encontrar las condiciones óptimas para la propagación in vitro de Alternanthera porrigens. Para las pruebas de desinfección y determinación del porcentaje de viabilidad de los explantes se utilizó etanol 70% con diferentes concentraciones de NaClO (1,0%, 1,5% y 2%) a distintos tiempos de exposición, obteniendo el menor porcentaje de contaminación utilizando etanol 70% y NaClO 2,0 % durante de 2 y 7 minutos respectivamente. Posteriormente, se realizaron ensayos de propagación in vitro en medios de cultivo Murashige y Skoog (MS) suplementado con reguladores de crecimiento 6-bencilaminopurina (BAP) y ácido 3-indol-acético (AIA) con el objetivo de evaluar los efectos en la elongación del tallo, generación de brotes (yemas axilares) bajo condiciones de luz durante 20 días a 20 ± 3°C. Se utilizaron concentraciones de 0, 0.1, 0.5 mg/l de AIA y 0, 0.5 y 1 mg/l de BAP donde el tratamiento con 0.5mg/l AIA y 1 mg/l BAP fue la combinación de fitohormonas que presentó resultados óptimos para la propagación. Por lo tanto, este trabajo puede presentarse como un protocolo reproducible para la propagación in vitro de Alternanthera porrigens.

Palabras clave: Alternanthera porrigens, brotes, propagación in vitro, reguladores de crecimiento, segmentos nodales.

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Introduction

The Amaranthaceae family includes many species that are used in traditional folk medicine for the treatment of numerous diseases that cause infections, inflammation and fever (Salvador & Días 2004) (Cai *et al*., 2003) (Gorinstein et al. 1991). The Amaranthaceae family has been recognized in Peru with 13 genera and 72 species (Brako & Zarucchi 1993) (Ulloa *et al*., 1993). As of 2006, eight species and two varieties in six genera were recognized as endemic to the desert scrub and meso-Andean regions (León *et al*., 2006). In addition, it has been studied for its pharmacological properties, among them the plants of the genus Alternanthera, which justifies its use in ethnopharmacology (Biella 2007).

Alternanthera porrigens (Moradilla) is distributed from 600 m in Arequipa to more than 3500 m elevations in several places in Peru; in the Salinas y Aguada Blanca National Reserve, it is found growing mainly on rocky and shrubby slopes (Macbride 1937). Its presence in the Lomas de Lúcumo is related to dispersal by cattle. It has also been found in the Lomas de Yuta, province of Islay, Arequipa (Quipuscoa *et al*., 2006) in the Cajamarca region and in the Loma Mongón, province of Casma, department of Ancash. It is widely used in traditional medicine, which attributes properties against inflammation, circulation, purgative, flu and menstrual cramps (Lleellish et al. 2015). Moradilla is commonly used to decrease pain in the uterus after childbirth (Bussmann *et al*., 2010), liver pain, influenza, kidney problems (Tene *et al*., 2007), inflammatory reactions, improve circulation, as a purgative, to attenuate influenza and menstrual disorders (Cerón 2006). In addition, toxicological studies indicate that it contains a good amount of essential oils, flavonoids, quinones and tannins, so it could be very useful in skin infection problems. Saponins and other substances are useful as an anti-allergic, anti-inflammatory, and antiseptic (Castillo 2019).

However, the increased demand for these plants, especially for food and medicinal purposes, is one of the causes of their rapid depletion of primary habitats (Boro *et al*., 1998). In addition, conventional propagation processes depend on the season. Therefore, they can only be achieved during humid and warm periods (Gnanaraj *et al*., 2011), being affected in their reproduction by unfavorable periods where they remain dormant or lose their leaves to produce flowers and fruits, thus hindering their recovery (Quipuscoa *et al*., 2006).

For this reason, the purpose of this research was to establish a method for disinfection of nodal segments and to use different concentrations of auxin (AIA) and cytokinin (BAP) to evaluate shoot generation and stem growth.

Materials and methods

Vegetable sample

The plant sample used in the research was collected in the district of Mollebaya, department of Arequipa, with UTM coordinates (WGS84) 19K 236418.472 8174306.538. The taxonomic classification was performed according to that proposed by the Angiosperm Phylogeny Group in "An update of the Angiosperm Phylogeny Group Classification for the orders and families of flowering plants: APG IV" (2016). The research was carried out at the Plant Biotechnology Laboratory of the Universidad Católica de Santa María (UCSM), Arequipa.

Conditions and preparation of the culture medium

Nodal segments were grown on MS medium (Murashige & Skoog) supplemented with 3% sucrose (w/v) and 7% agar (w/v), adding the growth regulators BAP and AIA, pH adjusted to 6.0 with 0.1 N NaOH or HCl, autoclaved at 121 °C for 20 min. All cultures were maintained under 16/8 h light/dark photoperiod with a light intensity of 2000 to 2500 Lux provided by fluorescent tubes and kept at a temperature of 20 ± 3°C.

Nodal segment disinfection trials

Nodal segments with vital morphological characteristics and free of pests were sampled and previously washed several times with potable water. For disinfection, 13 replicate trials were evaluated using 70% ethanol for 30, 60, 90, 120 and 150 seconds rinsing and NaClO at different concentrations 1.0%, 1.5% and 2.0% (v/v) for 5, 6, 7, 8 and 9 minutes rinsing, followed by 2 rinses with distilled water. Each explant was seeded in a test tube containing MS medium. Twenty test tubes were evaluated per test for 20

days. The values obtained from the disinfection tests were analyzed by ANOVA analysis of variance. Subsequently, the averages were separated by Tukey's test (0.05).

Effects of growth regulators on shoot induction and stem elongation.

Alternanthera porrigens explants were sown on MS medium supplemented with AIA and BAP at different concentrations (Table 2) with 3 replicates per treatment for a 20-day evaluation period.

Results and Discussion

The plant species collected were taxonomically determined in the facilities of the Herbarium of the Michael Owen Dillon Scientific Institute (IMOD), "Herbario Sur Peruano" (HSP) in the city of Arequipa, and correspond according to Table 1.

Plant disinfection tests

To determine the ideal conditions for surface sterilization of explants, 70% ethanol with different NaClO concentrations (1.0%, 1.5% and 2%) and exposure times were used. As shown in Table 1, the results revealed that nodal explants sterilized with 70% ethanol and 1.0% sodium hypochlorite for 30, 60, 90, 120, 150 s and 5, 6, 7, 8.9 min, respectively, had an average percentage of fungal contamination of 27%, bacterial of 39%, with yeasts of 21% and oxidation of 2%, with 11% of viable explants.

According to the analysis of variance for a 95% confidence level, it was found that there is no significant difference between the contamination percentages of the replicates by disinfection test ($p>0.05$). However, there are significant differences at 95% between one level and another of the disinfection tests generated by Tukey's significant difference test (HSD) (Figure 2).

This indicated that the concentration of the disinfecting agents may not have been adequate, favoring the proliferation of these microorganisms (Fig. 1-A, C, D). The study then proceeded with the 70% ethanol and 1.5% sodium hypochlorite tests for 90, 120 s and 7, 8, 9, respectively, revealing fungal contamination of 14.2%, bacterial contamination of 25%, a yeast contamination of 9.2% and oxidation of 8.3% and a survival rate of 43.3%. As shown, the percentage of contamination induced by fungi, bacteria and yeasts decreases considerably when the concentration of sodium hypochlorite is increased to 1.5% during the proposed times.

However, the percentage of explants showing oxidation increased by 6.3% for the tests using 1.0% hypochlorite, probably because the explant tissues were damaged during the preparation stage, evidencing a black or brown coloration as a consequence of the oxidation process due to phenolic compounds that are in large quantities in the vacuoles, mixed with the contents of the plastids and other organelles where polyphenol oxidases are confined (Preece & Compton 1991). These compounds are highly reactive and inhibit enzymatic activity, resulting in lethal darkening of explants (Fig. 1-B). Next, results were obtained with 70% ethanol and 2.0% sodium hypochlorite for 90, 120s and 5, 7 min, respectively. It was found that the nodal segments of A. porrigens, presented the lowest average percentage of fungal contamination (2.5%), bacterial (10%), and without contamination by yeasts and oxidation, thus obtaining 87.5% of viable explants. This is in agreement with that proposed by Alfaro *et al*. (2000), where for the reduction of the microbial population of medicinal plants, minimum doses of sodium hypochlorite are used, between 0.5 - 2.0%, and the immersion time should also be short, between 5 and 10 minutes. These disinfection tests led to the following experiments to evaluate the effects of growth regulators.

On the other hand, optimal results have been demonstrated with the use of disinfecting agents such as ethanol and sodium hypochlorite for the genus Alternanthera, as reported by Pikulthong *et al*. (2018), where the most suitable method for surface sterilization of meristematic tissues of Alternanthera sp. was with the use of 70% ethanol for 1 min and 3% sodium hypochlorite for 10 min, providing a contamination rate of 5% with the highest survival rate of 95%.

Effects of growth regulators on shoot induction and stem elongation

The results showed that nodal segments grown on MS media supplemented with AIA and BAP at different concentrations had significantly different stem lengths ($p<0.05$). However, as shown in Fig. 3 and Fig. 4, shoot generation (apical and axillary buds) had no difference between one treatment and another ($p>0.05$).

Treatment 4, MS medium supplemented with 0.5 mg/l AIA and 1.0 mg/l BAP, induced the highest average number of shoots with 5.7

shoots/explant, also presenting the highest average stem elongation 2.7 cm/explant followed by treatment 5 with 0.1 mg/l AIA and 1.0 mg/l BAP which induced the highest number of shoots and stem elongation with 4.3 shoots/explant and 2.2 cm/explant, respectively. Treatment 1, without using phytohormones, induced the lowest number of shoots with 2.3 shoots/explant and stem elongation 1.1 cm/explant (Table 2 and Figure 5). Reddish coloration in leaves and green stem of the explant was observed in all treatments. Good root induction was also observed in all treatments and increased leaf generation, especially in treatment 4. However, in contrast to root generation, treatments 1, 2 and 3 showed fewer leaves (data not shown).

According to work on the same species, Bennici and Schiff (1997) found that a high cytokinin:auxin ratio favors shoot regeneration in Amaranthus. Gnanaraj *et al*. (2011), used MS medium with different concentrations and combinations of plant growth regulators (BAP (0.5-2 mg/l), Kin (0.5-2 mg/l) and BAP (1.5 mg/l) + Kin (0.05-2 mg/l) 0.1 mg/l)) for the generation of multiple shoots from buds and nodal segments obtaining the highest percentage (94.3 ± 0,43 %) and maximum number (23.4 ± 0.38) of shoot induction on MS medium supplemented with 2.0 mg/l BAP from buds. The highest percentage (90.4 ± 0.82 %) and maximum number (15.2 ± 0.63) of shoot generation from nodal segments were observed on MS medium enriched with 1.5 mg/l BAP. In this work, it was found that the combination of auxin: cytokinin (0.5 mg/L: 1 mg/L) during 20 days of evaluation is in agreement with that proposed by Singh (2009), where they found maximum shoot generation of Alternanthera sessilis using MS medium supplemented with 1 mg/L AIA plus 1 mg/L BAP at 14 days of inoculation in the medium.

Conclusions

Test 13 of surface disinfection of nodal segments used to reduce bacterial, fungal, yeast and rust contamination was effective. The optimum conditions for the disinfection of nodal segments of A. porrigens were using 70% ethanol and 2.0% NaClO for 2 and 7 minutes of rinsing, respectively.

The appropriate concentrations of growth regulators during the in vitro multiplication stage of A. porrigens were 1.5 mg/L BAP and 1.5 mg/L AG3, where the number of shoots and aerial growth of the seedlings were observed.

For future research in vitro culture of Alternanthera porrigens, disinfecting agents such as 70% ethanol and 2.0% sodium hypochlorite are recommended because of their ease of acquisition, cost-effectiveness, efficacy against microorganisms and instantaneous action at low concentrations. In addition, in the evaluation with growth regulators, MS media supplemented with 0.5 mg/L AIA and 1.0 mg/L BAP is recommended for greater stem elongation and several shoots (apical buds and axillary buds).

Contribution information:

Jesús María Zambrano Salas performed methodology, supervision and project management; Keny Davi Alvarado Quiroz performed project management; Xiomara Bustinza carried out conceptualization, methodology, research and original drafting; Rosmery Cárdenas conducted research and original draft.

Conflict of interest information:

The corresponding author declares no conflict of interest on behalf of all authors.

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On research ethics and legal norms:

The research complied with all the established ethical norms in obtaining and handling the plant species to be studied, reagents and material used in the laboratory, the correct handling of the waste generated, and the corresponding treatment and recycling.

References

Alfaro T. CM, Rodríguez L., Rodríguez C., Ramos R., Carballo C., Cabezas C. 2000. Lavado y Desinfección de Drogas Vegetales. Normas Técnicas. Proceso Tecnológico (Proyecto). Departamento de Control Microbiológico del Centro de Investigación y Desarrollo de Medicamentos.

Bennici A, Schiff S. 1997. Micropropagation of Amaranthus (Amaranth). High-Tech and Micropropagation V: Springer. p. 20-9. DOI: https://doi.org/10.1007/978-3-662-07774-0_2

Biella CdA. 2007. Avaliação da atividade imunomoduladora de\'Alternanthera tenella\'Colla e investigação de ações do extrato aquoso em modelo de artrite experimental: Universidade de São Paulo. DOI: 10.11606/T.60.2007.tde-24092010-143641

Boro P, Sarma Deka A, Kalita M. 1998. Journal of Phytological Research. Clonal propagation of Alternanthera sessilis: A biopharmaceutically potent herbal medicinal plant. Vol. 2.; 103-106. Disponible en: https://jphytolres.org/paper/811

Brako L, Zarucchi JL. 1993. Catalogue of the flowering plants and gymnosperms of Peru: Missouri Botanical Garden. ISBN: 09-152-79193

Bussmann RW, Glenn A, Sharon D. 2010. Antibacterial activity of medicinal plants of northern Peru–can traditional applications provide leads for modern science?. Corpus ID: 55519247

Cai Y, Sun M, Corke H. 2003. Antioxidant activity of betalains from plants of the Amaranthaceae. Journal of agricultural and food chemistry. 51(8):2288-94. DOI: https://doi.org/10.1021/jf030045u

Castillo Vera H, Albán Castillo J, Castañeda R. 2019. Importancia cultural de la flora silvestre de la provincia de Cajabamba, Cajamarca, Perú. Arnaldoa. 26(3):1047-74. DOI: http://dx.doi.org/10.22497/arnaldoa.263.26313

Cerón Martínez Carlos E. 2006.Plantas medicinales de los Andes ecuatorianos In: Botánica Económica de los Andes Centrales. Universidad Mayor de San Andrés, La Paz, 2006: 285-293. Botánica Económica de los Andes Centrales 2006.pdf

Gnanaraj WE, ALIAS AJM, Subramanian KM, Nallyan S. 2011.Micropropagation of Alternanthera sessilis (L.) using shoot tip and nodal segments. https://www.sid.ir/en/Journal/ViewPaper.aspx?ID=216339

Gorinstein S, Arnao de Nue I, Arruda P. 1991. Alcohol-soluble and total proteins from amaranth seeds and their comparison with other cereals. Journal of agricultural and food chemistry. 39(5):848-50. DOI: https://doi.org/10.1021/jf00005a005

León B, Monsalve C, Sagástegui A, Sánchez I. 2006. Amaranthaceae endémicas del Perú. Revista Peruana de Biología. 13(2):31-2. Rev. peru biol. [online]. 2006, vol.13, n.2 [citado 2022-07-05], pp.31-32. Disponible en: <http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1727-99332006000200007&lng=es&nrm=iso>. ISSN 1727-9933.

Lleellish M, Odar J, Trinidad H. Guía de Flora de las Lomas de Lima. 2015. http://repositorio.serfor.gob.pe/handle/SERFOR/484

Macbride J. 1937. Convolvulaceae. Flora of Peru. Field Mus Nat Hist, Bot Ser. 13(6/2):321-83. <http://www.scielo.org.pe/scielo.php?script=sci_arttext&pid=S1727-99332006000200007&lng=es&nrm=iso>. ISSN 1727-9933

Pikulthong V, Phakpaknam S, Dechkla M, Teerakathiti T. 2018. Optimal medium for watercress (Alternanthera sp.) micropropagation. Suan Sunandha Science and Technology Journal. 5(2). t www.ssstj.sci.ssru.ac.th

Preece J, Compton M. 1991. Problems with explant exudation in micropropagation. High-Tech and Micropropagation. p. 168-89. https://doi.org/10.1007/978-3-642-76415-8_10

Quipuscoa Silvestre V, Tejada Pérez C, Fernández Ardiles C, Pauca Tanco A, Durand Vera K, Dillon MO. 2016. Diversidad de plantas vasculares de las Lomas de Yuta, provincia de Islay, Arequipa Perú, 2016. Arnaldoa. 23(2):517-46. DOI: https://doi.org./10.22497/arnaldoa.232.23207

Salvador MJ, Dias DA. 2004. Flavone C-glycosides from Alternanthera maritima (Mart.) St. Hil.(Amaranthaceae). Biochemical Systematics and Ecology. 1(32):107-10. DOI: 10.1016/S0305-1978(03)00180-7

Singh A, Kandasamy T, Odhav B. 2009. In vitro propagation of Alternanthera sessilis (sessile joyweed), a famine food plant. African Journal of Biotechnology. DOI: 10.5897/AJB09.307

Tene V, Malagon O, Finzi PV, Vidari G, Armijos C, Zaragoza T. 2007. An ethnobotanical survey of medicinal plants used in Loja and Zamora-Chinchipe, Ecuador. Journal of

ethnopharmacology. 111(1):63-81. DOI: https://doi.org/10.1016/j.jep.2006.10.032

Ulloa CU, Zarucchi JL, León B. 1993. Diez años de adiciones a la flora del Perú: 1993-2003. 2004. http://www.mobot.org/MOBOT/research/peru/pdf/DiezA%C3%B1osArnaldoa2004.pdf

ANNEXES

Table 1. Taxonomic classification of plant material collected.

Class	**Equitopsida**
Subclass	Magnoliidae
Order	Caryophyllales
Family	Amaranthaceae (Caryophyllaceae)
Genre	*Alternanthera*
Species	*Alternanthera porrigens* (Jacq.) Kuntze

According to the different tests, the percentage of viability and contamination of nodal segments of A. porrigens.

Test	**Method**	**Pollution**				**Viability**
		Mushrooms	**Bacteria**	**Oxidation**	**Yeast**	
P1	EtOH 70%, NaClO 1.0% (30 s, 5min)	35	40	0	25	0
P2	EtOH 70%, NaClO 1.0% (60 s, 6min)	30	35	0	30	5
P3	EtOH 70%, NaClO 1.0% (90 s, 7min)	25	40	5	20	10
P4	EtOH 70%, NaClO 1.0% (120 s, 8min)	25	40	0	15	20
P5	EtOH 70%, NaClO 1.0% (150 s, 9min)	20	40	5	15	20
P6	EtOH 70%, NaClO 1.5% (90 s, 7min)	20	30	5	15	30
P7	EtOH 70%, NaClO 1.5% (120 s, 7min)	15	40	5	15	25
P8	EtOH 70%, NaClO 1.5% (90 s, 8min)	20	25	10	5	40
P9	EtOH 70%, NaClO 1.5% (120 s, 8min)	10	20	10	10	50
P10	EtOH 70%, NaClO 1.5% (90 s, 9min)	10	10	15	5	60
P11	EtOH 70%, NaClO 1.5% (120 s, 9min)	10	25	5	5	55
P12	EtOH 70%, NaClO 2.0% (90 s, 5min)	5	15	0	0	80
P13	EtOH 70%, NaClO 2.0% (120 s, 7min)	0	5	0	0	95

Table 3. Effects of different concentrations of AIA and BAP on stem elongation and shoot induction of nodal segments of A. porrigens grown for 20 days.

No. Treatment	Concentration (mg/L)		Stem height (cm)	N° buds (pcs.)
	AIA	BAP		
T1	0.0	0.0	1.1	2.3
T2	0.5	0.0	1.5	2.7
T3	0.0	0.5	1.9	4.0
T4	0.5	1.0	2.7	5.7
T5	0.1	1.0	2.2	4.3

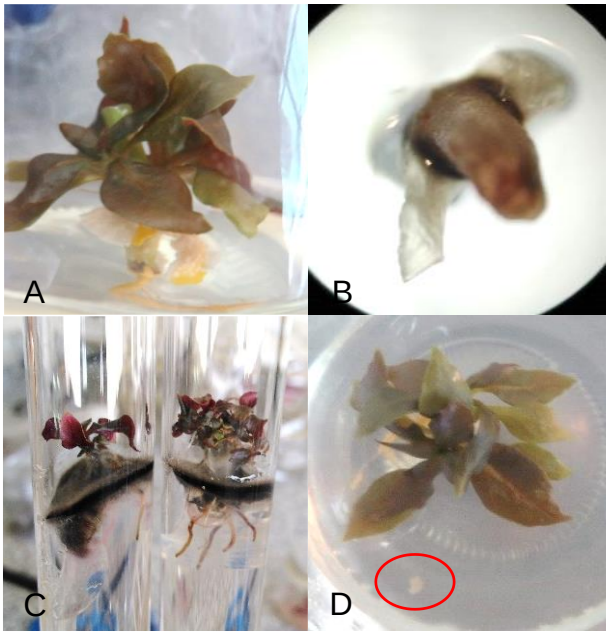


Figure 1. Nodal segments of A. porrigens exposed to different disinfection tests for 20 days. Explant contaminated by A) bacteria, C) fungi, D) yeasts and B) explants with the presence of rust.

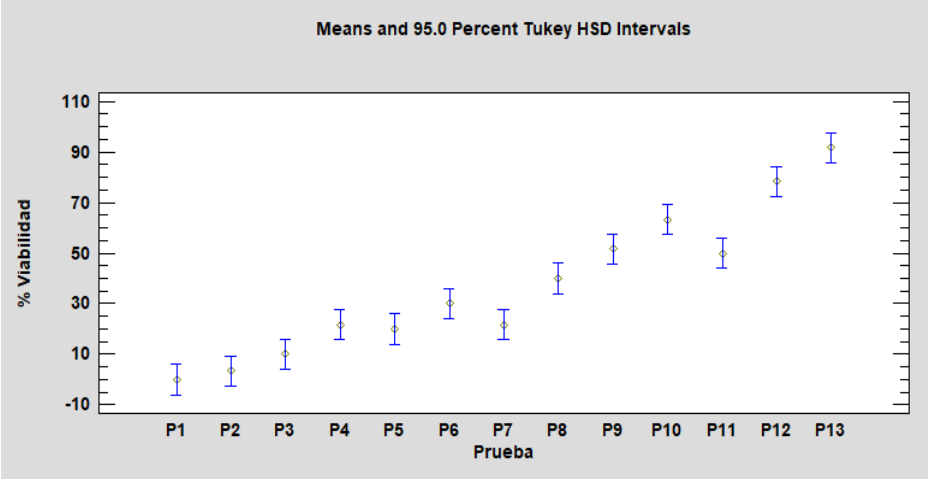


Figure 2. According to HSD, the viability of in vitro explants of A. porrigens with disinfection tests.

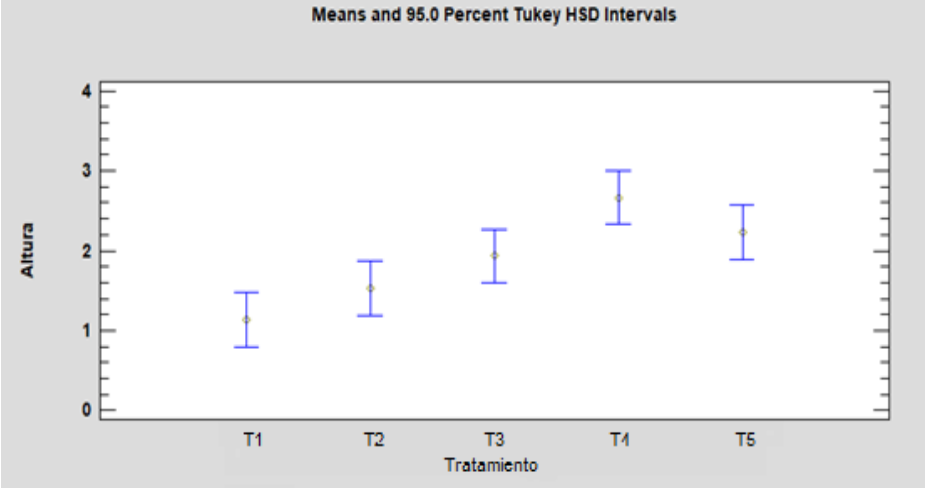


Figure 3. According to HSD, evaluate the stem elongation of A. porrigens explants at different concentrations of AIA and BAP.

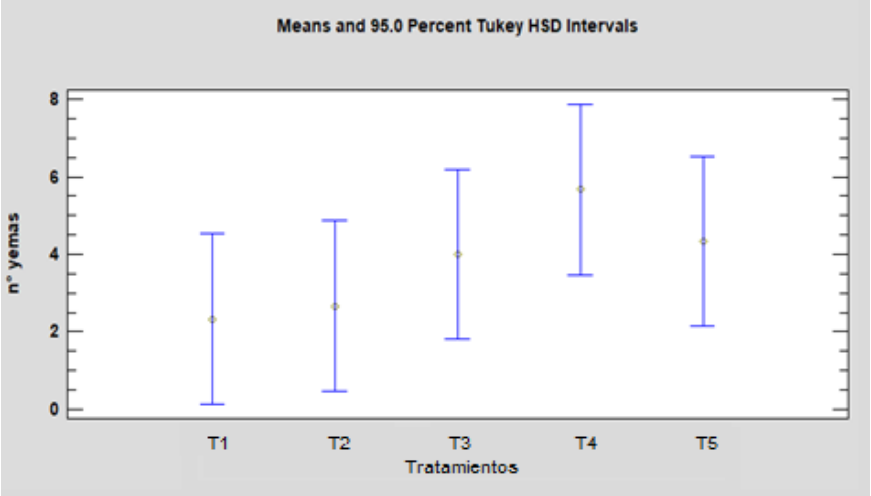
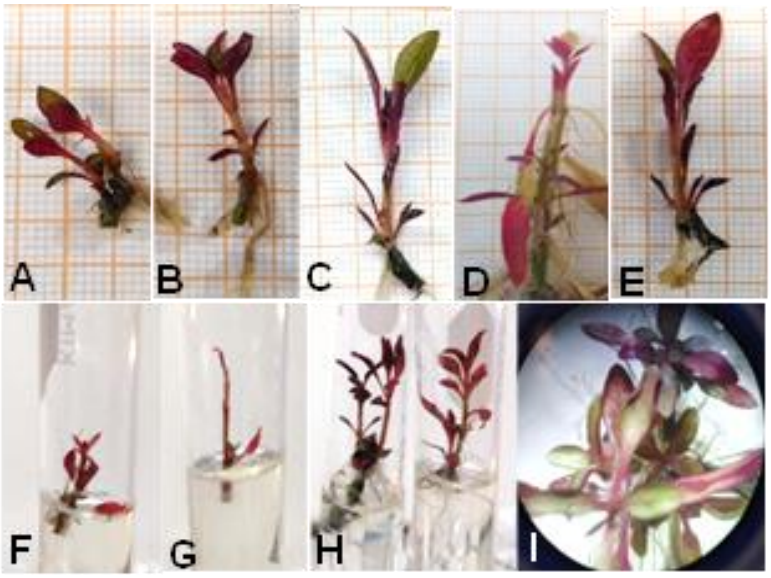


Figure 4. According to HSD, evaluate bud number induction of A. porrigens explants at different concentrations of AIA and BAP.



Effects of different concentrations of AIA and BAP on nodal segments of A. porrigens grown for 20 days. Stem elongation and shoot induction of explants according to treatments A) T1, B) and F) T2, C) and G) T3, D) and H) T4, E) T5. I) Explant exposed to the stereoscope for shoot counting.

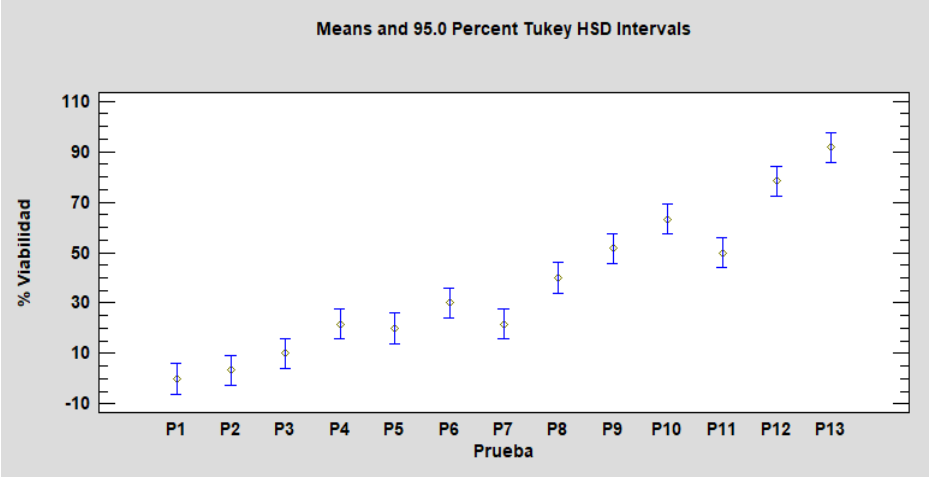


Figura 2. Evaluación de la viabilidad de explantes in vitro de A. porrigens con las pruebas de desinfección según HSD.

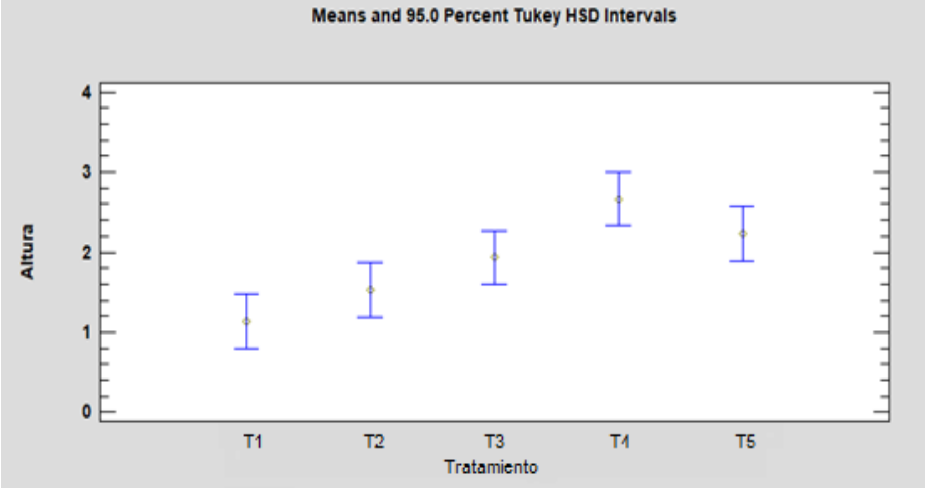


Figura 3. Evaluación de la elongación del tallo de explantes de A. porrigens a diferentes concentraciones de AIA y BAP según HSD.

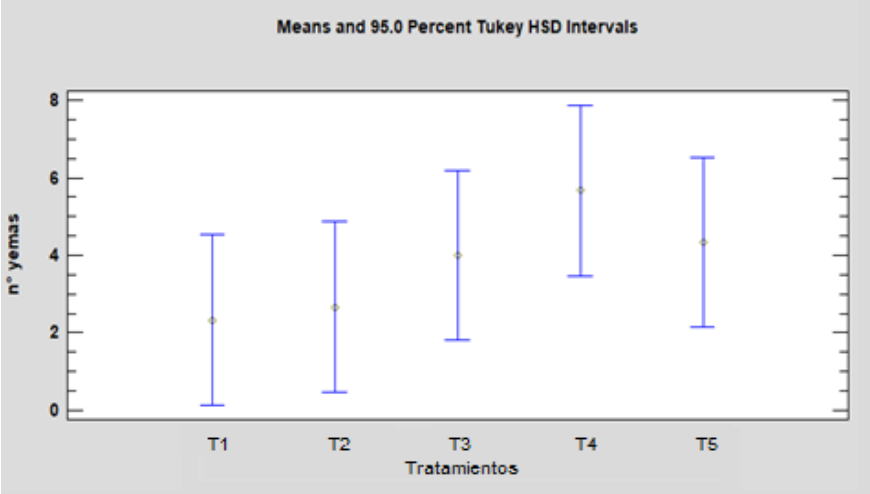


Figura 4. Evaluación de la inducción de numero de yemas de los explantes de A. porrigens a diferentes concentraciones de AIA y BAP según HSD.

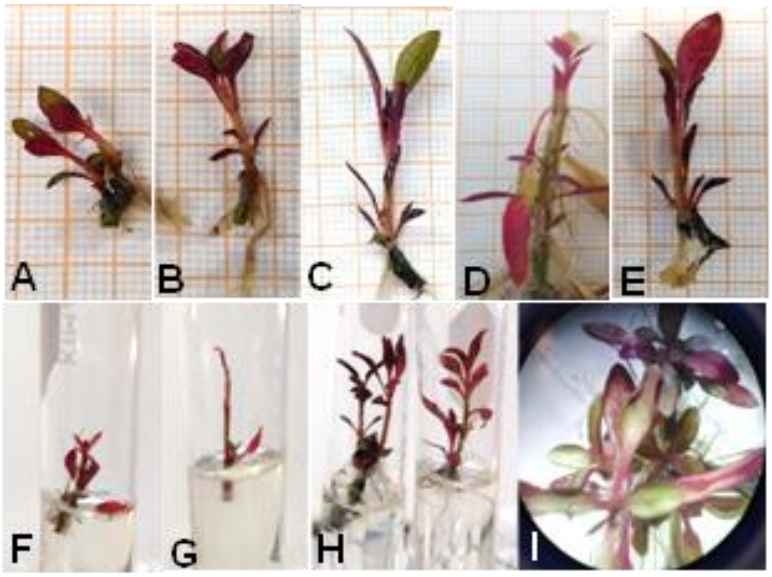


Figura 5. Efectos de las diferentes concentraciones de AIA y BAP de segmentos nodales de A. porrigens cultivados durante 20 días. Elongación del tallo e inducción de brotes de los explantes según los tratamientos A) T1, B) y F) T2, C) y G) T3, D) y H) T4, E) T5. I) Explante expuesto al estereoscopio para el recuento de brotes.