Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i5.3744

Article

Seed disinfection and culture media in the *in vitro* germination and growth of seedlings of *Eysenhardtia polystachya* (Ortega)

Norma Angélica Lorenzo-Barrera[1]
María Andrade-Rodríguez[1,§]
Oscar Gabriel Villegas-Torres[1]
Teresa de Jesús Rodríguez Rojas[2]
Erika Román Montes de Oca[2]

1 Posgrado en Ciencias Agropecuarias y Desarrollo Rural-Facultad de Ciencias Agropecuarias-Universidad Autónoma del Estado de Morelos. Av. Universidad 1001, Col. Chamilpa, Cuernavaca, Morelos, México. CP. 62209. Tel. 777 3297900. (norma.lorenzo@uaem.edu.mx; oscar.villegas@uaem.mx).

2 Universidad Autónoma del Estado de Morelos-Escuela de Estudios Superiores de Xalostoc. Nicolas Bravo s/n, Jaloxtoc, Morelos, México. Tel. 735 2802240. (teresa.rodriguez@uaem.mx; erika.romanm@uaem.edu.mx).

Autora para correspondencia: maria.andrade@uaem.mx.

Abstract

Eysenhardtia polystachya is a plant prized for the strength of its wood and medicinal use. It is propagated by seed; nevertheless, low germination and attack by insects and fungi in natural conditions limit its availability, which makes it highly vulnerable. *In vitro* culture allows the number of individuals to increase rapidly. The objective was to evaluate the effect of mineral salts of Murashige and Skoog and Woody Plant Medium culture media, both in concentration of 50, 75 and 100% (macro and micronutrients), in combination with two methods of seed disinfection. *E. polystachya* seeds collected in November 2021 in Tlayacapan Morelos were used; these were disinfected with silver nanoparticles, applied once or twice, and then seeded *in vitro*. The experimental design was completely randomized with factorial arrangement of treatments, with 10 replications of five seeds. Double application of silver nanoparticles generated 98.3% of aseptic seeds, compared to applying them once (86.7%). Plant height was also 34.5% higher. The MS medium generated better germination in the three concentrations (90 to 99%). Root length and dry matter were more abundant in the MS medium at 50%. It was concluded that it is convenient to use silver nanoparticles twice to disinfect *E. polystachya* seeds and to use the 50% MS culture medium for germination and seedling growth.

Keywords:

disinfection method, kidneywood, MS medium, WPM medium.

DOI: https://doi.org/10.29312/remexca.v16i5.3744

Introduction

The species *Eysenhardtia polystachya* (Ortega) has ecological potential due to its ability to establish itself in eroded soils, from plains to places with irregular topography, such as hills and mountainous ravines (Martín *et al*., 2021). Its wood is appreciated for its hardness and strength, so it is extracted for the construction of furniture, tools, posts for pens and fences, as well as firewood for homes. In addition, it is used in traditional medicine for the urinary and digestive systems and inflammations, both for human and for veterinary use (Martín *et al*., 2021; Lorenzo-Barrera *et al*., 2023).

It has several active ingredients that make it toxic to bacteria and fungi (Bernabé-Antonio *et al*., 2017). To extract the active ingredients, heartwood and sapwood are used; therefore, it is necessary to cut the stems, which in many cases causes the death of the plant; this has caused the reduction of natural populations (Beltrán-Rodríguez *et al*., 2017; Beltrán-Rodríguez *et al*., 2020). In addition to climate change, the current restricted distribution in wild conditions limits the availability of seeds and thus the spread; consequently, this species is highly vulnerable.

Seed quality is affected by several factors, such as purity, health, and germination aspects (Raya-Pérez *et al*., 2020). In *E. polystachya*, the seeds have a thin and impermeable testa, which can cause germination to be slow and heterogeneous and a low number of seedlings to be produced, causing natural populations not to regenerate quickly; likewise, under natural conditions, they are attacked by insects and fungi (Martín *et al*., 2021).

Authors such as Núñez-Cruz *et al*. (2018) observed germination variations in *E. polystachya*; they obtained 50% emergence in the germination chamber and 30% under natural conditions, without the effect of the pre-germination treatments applied (soaking and soaking + drying), and they point out that germination could be related to the degree of embryonic maturity, latency break, and the appropriate environmental conditions. Gelviz-Gelvez *et al*. (2020) evaluated seed germination with low water potentials without applying pre-germination treatments and obtained 66% germination.

Lorenzo-Barrera *et al*. (2024) studied *E. polystachya* under greenhouse conditions and obtained 34% germinated seeds, without applying treatment, 40% with soaking for 36 h and 96% with 600 ppm of gibberellic acid for 30 min. The use of micropropagation techniques in ornamental plants, plants of agri-food importance and forest species helps to rapidly increase the number of individuals with genetically stable plant material and free of pests and diseases through the cloning and growing of plants in controlled and aseptic conditions (Campos *et al*., 2020; Ramírez-Mosqueda *et al*., 2020; Bello-Bello and Spinoso-Castillo, 2022).

Likewise, through *in vitro* culture, it is possible to obtain extracts or secondary metabolites of plants of interest with pharmaceutical applications, using cell culture, without having to extract them directly from wild plants to avoid overexploitation and deforestation. In this sense, Bernabé-Antonio *et al*. (2017) used the hexane fraction of methanolic extracts obtained from cell suspension cultures of *E. polystachya* to inhibit the mycelial growth of *Rhizoctonia solani*.

In addition to the above, *in vitro* plant cell culture is a tool with the potential to accelerate the large-scale production of raw material in less time in a controlled manner at any time of the year, and yields of bioactive compounds can be higher than in wild plants (Haida *et al*., 2020; Bernabé-Antonio *et al*., 2021); Bernabé-Antonio *et al*. (2021) used *in vitro* culture of *Eysenhardtia platycarpa* in MS medium to culture suspension cells from internodal segments to obtain extracts and evaluate antifungal activity against *R. solani* and *Sclerotium cepivorum*.

Nonetheless, problems associated with contamination of culture media can occur in micropropagation, especially in the establishment phase of the *in vitro* culture, contamination that affects the growth of explants by competing for water, light, space, and essential nutrients (Campos *et al*., 2020; Ramírez-Mosqueda *et al*., 2020; Bello-Bello and Spinoso-Castillo, 2022).

This problem can be controlled by adding fungicides and antibiotics to the culture medium; however, due to the resistance of some strains, the use of such products is not recommended. Another alternative is the application of silver nanoparticles (AgNPs) (Ramírez-Mosqueda *et al*., 2020; Bello-Bello and Spinoso-Castillo, 2022), these have a broad microbicidal spectrum, do not generate

DOI: https://doi.org/10.29312/remexca.v16i5.3744

resistance as silver attacks a wide range of targets in microbes, are easy to acquire, are non-toxic when used in the correct dose, are inexpensive compared to other products, and have also been used to induce germination, increase yield, and promote the development of *in vitro* cultures (Spinoso-Castillo *et al*., 2017; Chávez-García *et al*., 2020; Bello-Bello and Spinoso-Castillo, 2022).

Based on the importance of kidneywood and the benefits that can be obtained with the use of *in vitro* culture techniques and contamination control, this research aimed to evaluate the effect of the concentration of mineral salts (50, 75, and 100%) of two culture media, MS (Murashige and Skoog, 1962) and WPM (McCown and Lloyd, 1981), in combination with two methods of seed disinfection to induce the greatest *in vitro* seed germination and seedling growth of *E. polystachya*.

Materials and methods

The study was carried out under controlled conditions in a micropropagation laboratory. The seeds that were used were collected from a wild tree in Tlayacapan, Mor. Mexico, 18° 95' 553" north latitude, 98° 98' 113" west longitude and altitude 1 640 m, in November 2021, and they were stored in a refrigerator at 4 °C. Three concentrations of mineral salts (50, 75, and 100%) of two culture media, MS (Murashige and Skoog, 1962) and WPM (McCown and Lloyd, 1981), without growth regulators, were evaluated. The media were supplemented with 3% sucrose, myoinositol 100 L, thiamine HCl, glycine, pyridoxine, and nicotinic acid (0.5 mg L^{-1} of each). The pH was set to 5.7 with a potentiometer (HANNA instruments, model HI8424).

Subsequently, 2 g L^{-1} of activated charcoal was added to prevent the negative effect of phenol exudation; 7 g L^{-1} of agar (Merk) was used. Twenty milliliters of culture medium were placed in 300 ml glass bottles. The medium was sterilized in a vertical autoclave (CV250) at 18 psi at 120 °C for 18 min. In the laminar flow hood (Novatech, model CFLH-120E), *E. polystachya* seeds were disinfected using two methods: 1) Roma detergent 0.2 g 100 ml^{-1}, stirring for 5 min, ethanol 96° at 70% (1 min), sodium hypochlorite (NaClO 0.9%) stirring for 5 min, silver nanoparticles (AgNPs) 75 µl 50 ml^{-1} (AgROVIT-CP), stirring for 10 min and 2) Roma detergent 0.2 g 100 ml^{-1}, stirring for 5 min, 96° ethanol at 70% (1 min), 75 µl 50 ml^{-1} AgNPs, stirring for 10 min, sodium hypochlorite (NaClO 0.9%) stirring for 5 min, and again AgNPs 75 µl 50 ml^{-1}, stirring for 10 min.

In each case, the seeds were rinsed three times with sterile distilled water and then established in the bottles with the respective culture medium (5 seeds per jar). The seeded bottles were placed in an incubation room at 24 °C with a photoperiod of 16 h with white fluorescent light (32 µE m^{-2} s^{-1}) and 8 h of darkness. The experimental design used was a completely randomized design with factorial arrangement of treatments, factor A: culture media at different concentrations; factor B: two ways to disinfect seeds. Ten replications per treatment were established, with five seeds per replication (culture bottle).

The following was evaluated in the initial stage: 1) germination variables: germination start (days), recorded when the radicle emerged, percentage of total germination at 30 days, and seed asepsis (%). 60 days after sowing, the following was recorded and 2) seedling growth variables: plant height (cm), root length, leaves per plant and dry matter per seedling (mg). The data were analyzed by variance (Anova) and Tukey's means comparison test ($p \leq 0.05$) was performed with the SAS® v. 9.2 statistical package.

Results and discussion

Seed asepsis and germination

The results of the analysis of variance indicated highly significant differences ($p \leq 0.01$) in seed asepsis due to the effect of the two factors studied and their interaction. The start of germination was affected only by the interaction of the concentration of mineral salts of culture media with disinfection methods ($p \leq 0.05$), whereas total germination was affected only by the culture media in which the seeds were established (Table 1).

DOI: https://doi.org/10.29312/remexca.v16i5.3744

Table 1. Percentage of aseptic seeds and germination due to the effect of the disinfection method in combination with culture media in *E. polystachya*.

Culture media (%)	Disinfection	Asepsis (%)	Total germination (%)
MS 100	1, AgNPs once	100 a	90 abc
MS 100	2, AgNPs twice	100 a	90 abc
MS 75	1, AgNPs once	90 b	98 ab
MS 75	2, AgNPs twice	100 a	100 a
MS 50	1, AgNPs once	90 b	94 abc
MS 50	2, AgNPs twice	100 a	84 bc
WPM 100	1, AgNPs once	70 d	98 ab
WPM 100	2, AgNPs twice	90 b	98 ab
WPM 75	1, AgNPs once	80 c	88 abc
WPM 75	2, AgNPs twice	100 a	96 abc
WPM 50	1, AgNPs once	90 b	82 c
WPM 50	2, AgNPs twice	100 a	86 abc
HSD (p# 0.05)		10.94	15.0
CV (%)		7.92	17.38

HSD= honestly significant difference; CV= coefficient of variation. Means with equal letters in each column are statistically equal according to Tukey's test ($p \leq 0.05$).

The use of silver nanoparticles (AgNPs) in the disinfection of *E. polystachya* seeds allowed the establishment of cultures without microorganisms. Asepsis was 100% when the AgNPs were added twice in the disinfection, with the medium MS at 100, 75 and 50%, WPM at 75 and 50%; however, when the MS was used at 100% and the seeds were disinfected with method 1, the culture was also completely aseptic (Table 1).

With disinfection method 1, 70, 80, 90, and 100% seeds free of contaminating microorganisms were obtained (Table 1). This indicates that using AgNPs twice during seed disinfection was the most appropriate way to establish the aseptic culture of this species.

The onset of germination occurred 3 and 4 days after sowing (das). According to the analysis of variance, there were no significant differences, so all media were adequate for the occurrence of the process of seed imbibition and thus the beginning of germination; in addition, the culture medium provided the necessary nutrients for the development of the plant (Andrade-Rodríguez *et al*., 2015) since the success of *in vitro* culture depends a lot on the composition of the culture medium used.

Except for orchids, mineral salts and supplements are not required for seed germination. Nevertheless, the concentration of mineral salts modifies the osmotic potential of the media and thus the availability of water for germination. In the same way, the type of medium and concentration of salts do not affect the contamination or percentage of aseptic cultures.

Total *in vitro* germination of *E. polystachya* seeds ranged from 82 to 100%, with an average of 92%. The 75% MS medium with disinfection method 2 generated the highest total germination (100%), which was statistically different only from what was obtained in the 50% MS and 50% WPM media, with the disinfection of method 2 and method 1, respectively (Table 1). Similarly, Bernabé-Antonio *et al*. (2021) obtained 98% germination of *Eysenhardtia platycarpa* seeds at 10 days of culture in MS medium; these researchers added four drops of Tween 20® to the sodium hypochlorite solution, and they point out that Tween is used as a surfactant agent, which may have helped to improve imbibition and thus increase the percentage of total germination.

Chávez-García *et al*. (2020) found that *in vitro* propagation is an alternative to conventional methods of propagation, as a seed with problems can germinate correctly because it increases the germination rate and the seed will be free of pathogens. The WPM medium generated good results

DOI: https://doi.org/10.29312/remexca.v16i5.3744

at the concentration of 100% with disinfection 1 and 2, and at 75% with disinfection 2 (98 and 96% germination, respectively); this medium presents a lower concentration of salts, NH_4^+ (5 mM), NO_3^- (9.7 mM), and Cl- (1.3 mM), than the MS medium (Andrade-Rodríguez *et al*., 2015; Campos *et al*., 2020), so that at 100%, it generated good total germination without having to dilute it.

Seedling growth

The analysis of variance showed a highly significant effect of culture media on the four seedling growth variables; in contrast, the seed disinfection method significantly affected only the height and dry matter of *E. polystachya* seedlings. There was also a significant effect of the interaction of the levels of the factors in the variables studied, hence the results are presented according to the combination of levels of the factors studied.

Seedling height was higher when seeds were sown in the MS culture medium at 100 and 75% (3.3 cm) in combination with disinfection method 2, in which AgNPs were applied twice, they were statistically equal to those of the MS medium at 50% and WPM at 100 and 50%, with disinfection method 2. The lowest growth in height was recorded in the 50% WPM medium with disinfection method 1 (Table 2), which was 1.3 cm less than the plants of the two best media.

Table 2. Growth variables of *E. polystachya* seedlings due to the effect of culture media in combination with the disinfection method, 60 days after sowing.

Culture media (%)	Disinfection	Height (cm)	Root length (cm)	Dry matter (mg)	Num. of leaves
MS 100	D1: AgNPs once	2.13 ef	4.24 d	41.3 bc	6 ab
MS 100	D2: AgNPs twice	3.37 a	5.42 cd	52.2 ab	7 a
MS 75	D1: AgNPs once	2.5 bcdef	5.06 cd	48.5 bc	5.5 bc
MS 75	D2: AgNPs twice	3.3 a	5.33 cd	46.3 bc	6.2 ab
MS 50	D1: AgNPs once	2.89 abcd	8.86 a	58.6 a	6.2 ab
MS 50	D2: AgNPs twice	3.23 ab	7.28 ab	44.3 bc	5.7 bc
WPM 100	D1: AgNPs once	2.31 cdef	6.6 bc	50.6 ab	5.8 bc
WPM 100	D2: AgNPs twice	3.19 ab	5.61 bcd	47 bc	5.8 bc
WPM 75	D1: AgNPs once	2.27 def	4.44 d	45.1 bc	5.7 bc
WPM 75	D2: AgNPs twice	2.85 bcde	5.29 cd	43.6 bc	5.8 bc
WPM 50	D1: AgNPs once	1.99 f	7.26 ab	44.0 bc	5.8 bc
WPM 50	D2: AgNPs twice	3.03 abc	5.6 bcd	39.6 c	5.8 bc
HSD (p# 0.05)		0.75	1.73	19.52	1.2
CV (%)		18.18	19.54	7.25	8.89

HSD= honestly significant difference; CV= coefficient of variation. Means with equal letters in each column are statistically equal according to Tukey's test ($p \leq 0.05$).

Any of the media used gave better results when combined with disinfection method 2. Pinedo-Panduro *et al*. (2022) mention that the MS medium is widely used and is suitable for most species, except for those most sensitive to salinity and in those cases, it can be used diluted to 75 and 50%. In kidneywood seeds, the three concentrations of the MS medium had the highest seedling height when combined with disinfection 2, which indicates the beneficial effect of AgNPs, which coincides with Chávez-García *et al*. (2020); Bello-Bello and Spinoso-Castillo (2022), who point out that these can increase yield and promote the development of *in vitro* cultures.

Contrary to height, seedling root growth was higher when seeds were grown in 50% MS culture medium in combination with disinfection methods 1 and 2 (8.86 and 7.28, cm respectively) and 50% WPM medium with disinfection method 1. The shortest root length was obtained when 100% MS and 75% WPM media were used, both combined with disinfection method 1, which had 4.63 and 4.42 cm less root length than those with longer roots (Table 2).

DOI: https://doi.org/10.29312/remexca.v16i5.3744

In general terms, the media that had plants with lower height originated greater root length. The components of the culture medium along with the application of the disinfectant affected both the height and root growth. In this regard, Bello-Bello and Spinoso-Castillo (2022) mention that the application of silver nanoparticles at low concentrations helps stimulate development, break dormancy, or promote seed germination and growth of some species.

Root size is important during the phase of acclimatization and establishment in soil; on this point, Pinedo-Panduro *et al*. (2022) report that, in order for micropropagated plants to survive the process of acclimatization and transplantation to the final field, it is necessary for them to have a good number and size of roots. However, it should be noted that seedlings with longer roots hinder the process of transplanting to substrate during the passage to the acclimatization phase.

Plant growth is also assessed by the accumulation of dry matter. In this research, the highest amount of dry matter was produced in seedlings grown in 50% MS medium (58.6 mg) with disinfection method 1, followed by those grown in 100% MS (52.2 mg) with disinfection 2 and by those grown in 100% WPM medium with disinfection method 1. The opposite was observed in the seedlings obtained from seeds grown in the WPM medium at 50% with disinfection method 2, since only 39.6 mg dry matter was obtained, 19 mg less than in the best treatment.

The seedlings of the other treatments generated similar amounts of dry matter (Table 2). Martínez-Villegas *et al*. (2015) mention that the accumulation of dry matter depends on the composition of the culture medium and the species that is being grown since, in *Euphorbia leucocephala*, the highest accumulation of dry matter (92.2 mg per plant) was obtained in the plants grown in the modified WPM medium; therefore, it is important to choose the appropriate culture medium for the species of interest and thus avoid any type of physiological or growth problem in plants grown *in vitro*.

The number of leaves per seedling showed few differences due to the effect of the treatments. The culture media of 100% MS with seeds disinfected with method 2, 75% MS with disinfection 2, and 50% MS with disinfection 1 were the ones that generated the highest number of leaves per seedling (6 and 7 leaves) (Table 2). The concentration of nutrients in the MS medium was adequate to supply water and nutrients to the kidneywood seeds; this medium contains high concentrations of ammonium NH_4^+ ions (20.6 mM), nitrate NO_3^- ions (39.4 mM), chlorine Cl^- ions (6 mM), and MoO_4^- (1 mM) (Martínez-Villegas *et al*., 2015) and did not have a toxic effect on plants.

The characteristics that give *in vitro* plants greater chances of surviving during acclimatization in greenhouses are the obtaining of a good size and foliage because these two parameters are related to greater photosynthetic capacity, which allow the plants to perform better radiation capture and therefore, greater accumulation of photosynthates (Pinedo-Panduro *et al*., 2022). Therefore, *E. polystachya* developed a good number of leaves during the 60 days after sowing.

Conclusions

Using the double application of AgNPs in the disinfection of *E. polystachya* seeds reduced the presence of fungi and bacteria in the culture bottles, which generated the highest asepsis, height, and dry matter of the seedlings. The MS culture medium gave better results in seed germination and seedling growth; it generated greater root length and dry matter in a concentration of 50%.

Acknowledgements

To the National Council of Science and Technology (CONACYT), for its acronym in Spanish for the scholarship awarded to the first author with scholarship number 665515. To the Faculty of Agricultural Sciences and the Autonomous University of the State of Morelos for their support to conduct the research.

DOI: https://doi.org/10.29312/remexca.v16i5.3744

Bibliography

1 Andrade-Rodríguez, M.; Vargas-Araujo, J.; Villegas-Torres, O. G.; López-Martínez, V.; Guillen-Sánchez, D. y Alia-Tejacal, I. 2015. Germinación de semillas y crecimiento de plántulas de Cattleya (*Brassolaeliocattleya*) *in vitro*. Interciencia. 40(8):549-553. https://www.interciencia.net/wp-content/uploads/2017/10/549-c-andrades5.pdf.

2 Bello-Bello, J. y Spinoso-Castillo, J. 2022. Utilización de nanopartículas de plata en la micropropagación de plantas. Revista Interdisciplinaria en nanociencias y nanotecnología. 16(30):1-14. https://doi.org/10.22201/ceiich.24485691e.2023.30.69692.

3 Beltrán-Rodríguez, L.; Manzo-Ramos, F.; Maldonado-Almanza, B.; Martínez-Ballesté, A. and Blancas, J. 2017. Wild medicinal species traded in the Balsas Basin, Mexico: risk analysis and recommendations for their conservation. Journal of Ethnobiology. 37(4):743-764. https://doi.org/10.2993/0278-0771-37.4.743.

4 Beltrán-Rodríguez, L.; Maldonado-Almanza, B.; Cristians, S.; Blancas, J.; Sierra-Huelz, A. y Bye, R. 2020. Las cortezas como productos forestales no maderables en México: Análisis nacional y recomendaciones para su aprovechamiento sostenible. Instituto de Biología. Universidad Nacional Autónoma de México (UNAM). Consejo Nacional de Ciencia y Tecnología. 47 p.

5 Bernabé-Antonio, A.; Maldonado-Magaña, A.; Ramírez-López, C. B.; Salcedo-Pérez, E.; Meza-Contreras, J. C.; González-García, Y.; López-Dellamary, T. and Cruz-Sosa, F. 2017. Establishment of callus and cell suspension cultures of *Eysenhardtia polystachya* (Ortega) and fungistatic activity of their extracts. South African Journal of Botany. 112:40-47. https://doi.org/10.1016/j.sajb.2017.05.023.

6 Bernabé-Antonio, A.; Sánchez-Sánchez, A.; Romero-Estrada, A.; Meza-Contreras, J. C.; Silva-Guzmán, J. A.; Fuentes-Talavera, F. J.; Hurtado-Díaz, I. and Cruz-Sosa, F. 2021. Establishment of a cell suspension culture of *Eysenhardtia platycarpa*: Phytochemical screening of extracts and evaluation of antifungal activity. Plants. 10(2):1-21. https://doi.org/10.3390/plants10020414.

7 Campos, R. J.; Arteaga, M. C.; Campos, S. R.; Chico, J. R. y Cerna, R. L. 2020. Establecimiento de un protocolo de desinfección y micropropagación *in vitro* de "caoba" *Swietenia macrophylla* King (Meliaceae). Arnaldoa. 27(1):141-156. http://doi.org/10.22497/arnaldoa.271.27107.

8 Chávez-García, J. A.; Andrade-Rodríguez, M.; Bello-Bello, J. J.; Rueda-Barrientos, M. C.; Guillén-Sánchez, D. y Sainz-Aispuro, M. J. 2020. Nanopartículas de plata en el establecimiento *in vitro* de ápices de gladiolo. Revista Fitotecnia Mexicana. 43(4-A):557-564. https://doi.org/10.35196/rfm.2020.4-A.557.

9 Gelviz-Gelvez, S. M.; Pavón, N. P.; Flores, J.; Barragán, F. y Paz, H. 2020. Germinación de siete especies de arbustos en el centro semiárido de México: efecto de la sequía y el tamaño de la semilla. Botanical Sciences. 98(3):464-472. http://doi.org/10.17129/botsci.2537.

10 Haida, Z.; Nakasha, J. J. and Hakiman, M. 2020. *In vitro* responses of plant growth factors on growth, yield, phenolics content and antioxidant activities of *Clinacanthus nutans* (Sabah Snake Grass). Plants. 9(8):1-17. https://doi.org/10.3390/plants9081030.

11 Lorenzo-Barrera, N. A.; Andrade-Rodríguez, M.; Villegas-Torres, O. G.; Román-Montes, E.; Sotelo-Nava, H.; Rodríguez-Rojas, T. J. y Suárez-Rodríguez, R. 2023. Usos del palo dulce *Eysenhardtia polystachya* (Ort.) Sarg., en cuatro municipios del estado de Morelos, México. Polibotánica. 55(28):161-177. http://doi.org/10.18387/polibotanica.55.11.

12 Lorenzo-Barrera, N. A.; Andrade-Rodríguez, M.; Villegas-Torres, O. G. and Sotelo-Nava, H. 2024. Pre-germination treatments on kidneywood (*Eysenhardtia polystachya*) seeds. Revista Ciência Agronômica. 55:e20238765. https://doi.org/10.5935/1806-6690.20230067.

DOI: https://doi.org/10.29312/remexca.v16i5.3744

13 McCown, B. H. and Lloyd, G. 1981. Woody Plant Medium (WPM)-a mineral nutrient formulation for microculture of Woody Plant Species. HortScience. 16(4):453-453.

14 Martín, R. M. H.; Ibarra, F. A.; Moreno, M. S.; Hernández, J. E. y Retes, R. 2021. Costo beneficio asociado con la cosecha de semilla de palo dulce y sitiporo en la región central de Sonora, México. Revista Mexicana de Agronegocios. 48(1):754-764. https://www.redalyc.org/articulo.oa?id=14167610015.

15 Martínez-Villegas, Y. M.; Andrade-Rodríguez, M.; Colinas-León, T.; Villegas-Torres, O. G.; Castillo-Gutiérrez, A. y Alia-Tejacal, I. 2015. Efecto de las sales inorgánicas del medio de cultivo en el crecimiento de pascuita (*Euphorbia leucocephala* Lotsy). Revista Fitotecnia Mexicana. 38(4):369-374. https://www.scielo.org.mx/scielo.php?pid=S0187-73802015000400004&script=sci-abstract&tlng=pt.

16 Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco cultures. Physiology Plantarum. 15(3):473-497.

17 Núñez-Cruz, A.; Meave, J. A. and Bonfil, C. 2018. Reproductive phenology and seed germination in eight tree species from a seasonally dry tropical forest of Morelos, Mexico: Implications for community-oriented restoration and conservation. Tropical Conservation Science. 11(1):1-14. https://doi.org/10.1177/1940082917749946.

18 Pinedo-Panduro, M.; Alves-Chagas, E.; Freitas-Luz, F.; Cardoso-Chagas, P.; Panduro-Tenazoa, N. M.; Bardales-Lozano, R.; Abanto-Rodríguez, C.; Paredes-Dávila, E. y Collazos-Saldaña, H. 2022. Efecto de las sales de los medios de cultivo Murashige & Skoog y Knudson sobre el establecimiento *in vitro* de *Epidendrum schomburgkii* Lindl. Ciencia y Tecnología Agropecuaria. 23(3):2526, 1-14. https://doi.org/10.21930/rcta.vol23-num3-art:2526.

19 Ramírez-Mosqueda, M. A.; Sánchez#Segura, L. and Hernández#Valladolid, S. L. 2020. The influence of silver nanoparticles on a common contaminant isolated during the establishment of *Stevia rebaudiana* Bertoni culture. Plant Cell Tissue and Organ Culture. 143(3):609#618. https://doi.org/10.1007/s11240#020#01945#9.

20 Raya-Pérez, J. C.; Aguirre-Mancilla, C. L.; Covarrubias-Prieto, J.; Ramírez-Pimentel, G. y Iturriaga, G. 2020. El osmoacondicionamiento de las semillas agrícolas. Ciencia y Tecnología Agropecuaria. 8(1):1-8. https://www.somecta.org.mx/rev-ciencia-y-tecnol-agrop-mexico/revistas/2020-1/2020-1-1/.

21 SAS Institute Inc. 1995. SAS/STAT User's guide, Version 9,2. SAS Institute. Cary, NC.

22 Spinoso-Castillo, J. L.; Chávez-Santoscoy, R. A.; Bogdanchikova, N.; Pérez-Sato, J. A.; Morales-Ramos, V. and Bello-Bello, J. J. 2017. Antimicrobial and hormetic effects of silver nanoparticles on *in vitro* regeneration of vanilla (*Vanilla planifolia* Jacks. ex Andrews) using a temporary immersion system. Plant Cell Tissue and Organ Culture. 129(2):195-207. https://doi.org/10.1007/s1124001711698.

Revista Mexicana de Ciencias Agrícolas

DOI: https://doi.org/10.29312/remexca.v16i5.3744

Seed disinfection and culture media in the *in vitro* germination and growth of seedlings of *Eysenhardtia polystachya* (Ortega)

Journal Information
Journal ID (publisher-id): remexca
Title: Revista mexicana de ciencias agrícolas
Abbreviated Title: Rev. Mex. Cienc. Agríc
ISSN (print): 2007-0934
Publisher: Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias

Article/Issue Information
Date received: 01 February 2025
Date accepted: 01 April 2025
Publication date: 06 August 2025
Publication date: Jul-Aug 2025
Volume: 16
Issue: 5
Electronic Location Identifier: e3744
DOI: 10.29312/remexca.v16i5.3744
Funded by: Consejo Nacional de Ciencia y tecnología
Funded by: Universidad Autónoma del Estado de Morelos
Award ID: 665515

Categories

Subject: Articles

Keywords:

Keywords:

disinfection method
kidneywood
MS medium
WPM medium

Counts

Figures: 0
Tables: 2
Equations: 0
References: 22
Pages: 0