

Pre-germination treatments on kidneywood (*Eysenhardtia polystachya*) seeds¹

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ABSTRACT - Kidneywood (*E. polystachya*) is a medicinal plant used for urinary and digestive system infections and inflammations in humans and animals. In addition, kidneywood is used as fodder and its wood is employed in rural housing construction. Populations of this plant have been reduced by the extraction that occurs in its natural habitat; in addition, the germination of its seeds is slow. To improve its germination, this research aimed to evaluate the effect of pre-germination treatments on seed germination and seedling growth of *E. polystachya*. Seven pre-germination treatments and a control were evaluated in a completely randomized experimental design with four replicates per treatment and 25 seeds per replicate. The results indicated that all treatments applied to the seeds produced higher germination percentages (100 to 182%) and greater seedling growth (14,6 to 59,8% more in height) than the control, with the exception of soaking in water. The pretreatment that generated the best response was the application of AG₃ at 600 ppm for 30 min, with 96% germination, 19,4 cm height, 3,9 mm stem diameter, 20 cm root length, 21 leaves per plant and 375 mg dry matter per seedling. Mechanical scarification generated 86% germination, 16,1 cm height, 2,9 mm stem diameter, 18 cm long roots, 12,5 leaves, and 354 mg dry matter, added to which it initiated emergence in less time (3,7 d), also making it a good and less costly option.

Key words: Germination. Seed treatment. Scarification. AG₃.

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INTRODUCTION

Eysenhardtia polystachya (Ortega) is a deciduous shrub 3 to 6 m tall in the Fabaceae family; it is commonly known as palo dulce, palo azul or ursa in Mexico (CONABIO, 2020), but as kidneywood in the United States. It is distributed from Arizona, USA, to Oaxaca, Mexico. Its distribution ranges from pine-oak forests to lowland rainforests, at elevations from 760 to 1802 m. It has great ecological potential due to its ability to establish itself in areas with eroded soils and irregular topography (DURÁN; SOUSA, 2013).

E. polystachya has use value in traditional medicine for urinary, digestive and inflammatory ailments, both for humans and domesticated animals (PÉREZ; GARCÍA, 2014). The antibiotic properties are attributed to the fact that they contain a large amount of active principles such as polyphenolic compounds, which are toxic to several microorganisms (BERNABÉ-ANTONIO *et al.*, 2017). This tree is also used as a building material for rural housing and as fodder for domesticated animals (CASTAÑEDA *et al.*, 2019; GUAL, 2018). For the above uses, the plant is extracted from its natural habitat, which causes a decrease in its population, since there is no history of cultivation for medicinal or timber purposes.

E. polystachya is propagated by seeds, which are essential for the survival of this species (CONABIO, 2020) since to date there are no reports of any other propagation method. Seed quality is affected by several factors such as purity, health and germination aspects. In addition, germination and seedling establishment in the field are essential phases in the life cycle of plants and are affected by the amount of reserves accumulated during their formation and the efficiency with which they are used in the development process (RAYA-PÉREZ *et al.*, 2020). In kidneywood, 58,49% germination was observed under laboratory conditions in Petri dishes, with germination beginning at 8,6 days (CAMACHO, 1987). Similarly, González and Camacho (2000) obtained 58% germination in gravel with a particle size of 1-4 mm. The effect of water potential on the seeds was also evaluated and 35% germination was obtained with 0,6 MPa (GELVIZ-GELVEZ *et al.*, 2020). The seeds of this species have a water-impermeable testa, which results in low, slow, heterogeneous germination and a low number of seedlings, making it difficult for natural populations to regenerate. This background motivated the development of this research to improve the germination of the seeds of this species.

The low germination of this species may be related to physiological aspects such as dormancy, a condition that prevents the germination of viable seeds, even if they are in ideal conditions to do so

(moisture, temperature and oxygen concentration) (GONZÁLEZ-AMAYA *et al.*, 2018). For some species, procedures have been used that help to reduce this physiological condition, making germination faster and more uniform; among these are the application of hydrothermal treatments (hot water), mechanical scarification, chemical scarification (sulfuric acid, nitric acid), and germination stimulators (gibberellic acid) at different concentrations and times (FLORES *et al.*, 2020; MERINO-VALDÉS *et al.*, 2018). However, osmoconditioning or metabolic conditioning treatments are not universal for all species; they may work for some species of a genus, but do not necessarily work for all (CANO-VÁZQUEZ *et al.*, 2015; RAYA-PÉREZ *et al.*, 2020).

Therefore, this research aims to evaluate the effect of pre-germination treatments on seed germination and seedling growth of *E. polystachya* to define the treatment that allows greater germination for plant production in order to be used in the renewal of populations and to preserve this valuable resource.

MATERIAL AND METHODS

The study was conducted in a greenhouse belonging to the Faculty of Agricultural Sciences of the Autonomous University of the State of Morelos, located at a latitude of 18°98'27"- 18°86'42" N, longitude of 99°23'18"- 99°94'15" W and an elevation of 1540 m, during the months of January to June 2021; temperatures ranged from 28 to 33 °C.

E. polystachya pods from Tlayacapan, Morelos, Mexico were used; they were collected in their natural environment in November 2020. Seeds without apparent mechanical damage, without spots and without traces of insect attack (holes or larvae) were selected. For seed preparation, the pods were washed with 5% commercial chlorine bleach for 5 min to disinfect without causing damage to the seed. They were rinsed twice, placed on absorbent paper and left to dry in shaded conditions. One hundred seeds were used per treatment.

Eight pre-germination treatments were applied: 1) Control (untreated seeds), 2) Soaking in distilled water (50 mL) for 36 h (at a room temperature of 27 °C), 3) Mechanical scarification with 120-grit wood sandpaper (the seed testa was sanded until the embryo was observed), 4) 5% hydrogen peroxide (H₂O₂) (50 mL) for 30 min, 5) gibberellic acid (AG₃) at four concentrations (150, 300, 450 and 600 ppm) (50 mL) for 30 min. Two rinses were performed after removing the H₂O₂ and AG₃. After treatment, seeds were placed in 100-cavity black plastic seedbeds with Sunshine Mix #3® substrate, and were

established in a covered space with transparent plastic and anti-aphid mesh on the walls. Daily irrigation was applied up to the drip point in the seedbed.

A completely randomized experimental design was used, with four replicates per treatment and 25 seeds per replicate. The number of germinated seeds was counted daily. The germination evaluation was concluded 33 days after sowing (das), when emergence was no longer observed. The variables evaluated were the onset of seedling emergence (days) and total emergence (%).

At 150 days after sowing, seedling growth variables were evaluated: stem diameter (mm), seedling height (cm), leaves per seedling (No.), and total dry matter (g). For this last variable, 10 seedlings per replicate were taken and placed in a drying oven at 80 °C for 48 h, and the dry matter weight was subsequently determined.

Data were studied by means of analysis of variance (ANOVA) and for the variables with treatment effect, Tukey's multiple comparison test ($p \leq 0,05$) was applied. The analysis procedures were performed with the SAS® v. 9,2 statistical package.

RESULTS AND DISCUSSION

The analysis of variance showed a highly significant effect ($P \leq 0,01$) of the evaluated treatments on the germination and seedling growth variables, indicating a different response of the *E. polystachya* seeds due to the effect of the pre-germination treatment applied to each group of seeds (Table 1).

Seed germination

The onset of emergence occurred at 3,7 days after sowing in the seeds that were scarified; in contrast, the control took 15 days (Figure 1). Testa attrition allowed

faster imbibition, interrupting dormancy. This agrees with Bárcenas-Argüello *et al.* (2013) who indicate that dormancy interruption is induced by scarification abrasion, which favored all three *Cephalocereus* species to initiate establishment in a shorter period of time.

Treatments that remove or degrade the testa or endocarp are adequate to achieve faster germination because they reduce the mechanical resistance of the testa and allow imbibition to occur faster, thereby interrupting dormancy. The water presses the physical barrier of the macrosclereids and the intercellular spaces remain connected, facilitating the contact of the water with the embryo and stimulating its development so that the radicle breaks the barrier and emergence occurs (MARTÍNEZ-CALDERÓN *et al.*, 2020; VIVEROS *et al.*, 2015).

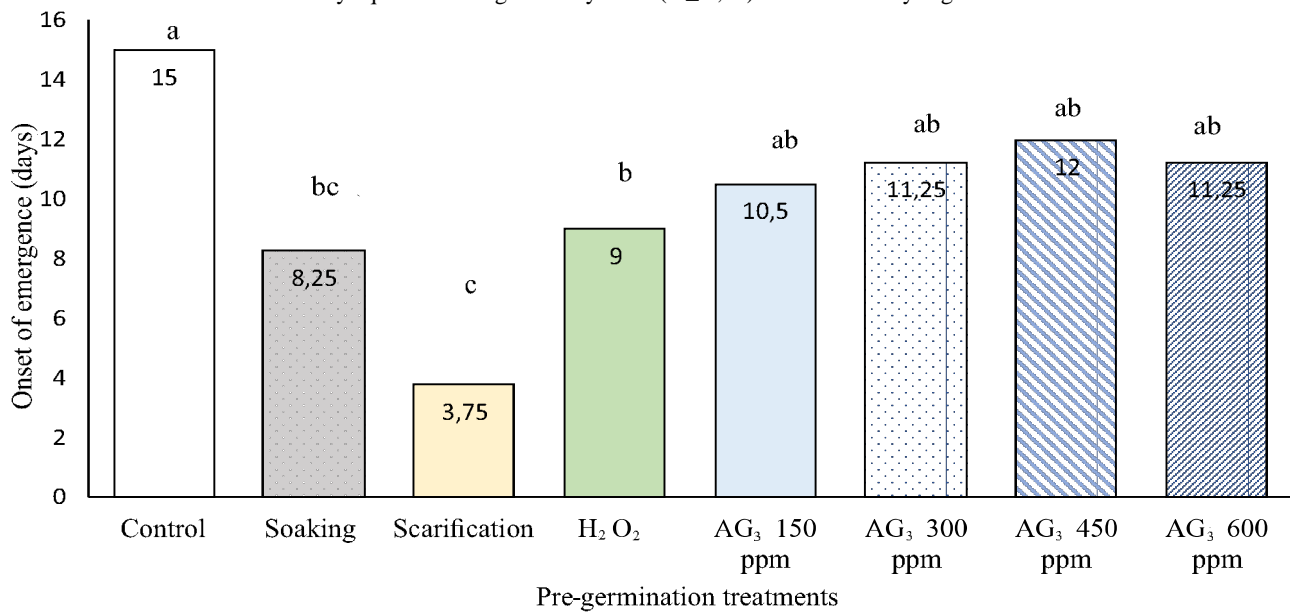
Soaking with water and application of hydrogen peroxide to *E. polystachya* seeds resulted in the onset of emergence at 8 and 9 days, respectively (Figure 1). These pretreatments were the second fastest in promoting imbibition in the first days and radicle emergence was detected at the end of the first week. This can be attributed to the softening induced by water on the seed and to reaching an ideal moisture level for the imbibition phase to occur. After that, enzymatic, biochemical and physiological processes begin, such as respiration, protein hydration, subcellular structural changes, and cell elongation, among others, which contribute to the onset of seedling emergence (MERINO-VALDÉS *et al.*, 2018; RAMÍREZ-VILLALOBOS *et al.*, 2017). Such changes depend on the type of seed and the conditions of exposure to osmoconditioning (CANO-VÁZQUEZ *et al.*, 2015; MERINO-VALDÉS *et al.*, 2018; RAYA-PÉREZ *et al.*, 2020). This may also be related to the washing or removal of germination inhibitors such as abscisic acid (ABA) that accumulate in the seed coats and induce dormancy (CAMACHO, 1987).

Table 1 - Mean squares and statistical parameters of ANOVA for germination and seedling growth variables due to the effect of pre-germination treatments on *E. polystachya*

SV	DF	OE	TE	Height	SD	RL	Leaves	DM
Treatments	7	1124**	57478**	544,402**	0,3941**	389,424**	1173,983**	0,0780**
Error	24	3,31	29,0	2,083	0,00038	0,571	2,272	0,000066
R ²		0,75	0,94	0,69	0,90	0,85	0,82	0,91
CV (%)		18,2	7,4	9,6	7,66	4,72	10,48	2,51
Average		10,03	72,68	14,9	2,5	15,9	14,38	0,324

SV: Source of variation, DF: Degrees of Freedom, CV: Coefficient of Variation, R²: Coefficient of Determination, OE: Onset of emergence, TE: Total emergence, SD: Stem diameter, RL: Root length, DM: Dry matter, **: highly significant effect ($P \leq 0,01$)

Figure 1 - Onset of emergence of *E. polystachya* seedlings whose seeds received eight pre-germination treatments. HSD= 4,5. Means with the same letter are statistically equal according to Tukey's test ($P \leq 0,05$). HSD=Honestly Significant Difference



In holywood (*Guaiacum sanctum* L.), similar results were observed in the onset of seedling emergence when seeds were soaked in water or hydrogen peroxide. The authors concluded that H₂O₂ is effective for species with dormancy problems (MEX *et al.*, 2021).

Abscisic acid increases during fruit ripening and may be a factor that induces dormancy by counteracting the effects of gibberellins. This compound can sometimes be removed with water; however, the disappearance of ABA does not necessarily coincide with the onset of germination (MEX *et al.*, 2021; RAMÍREZ *et al.*, 2012; RAMÍREZ-VILLALOBOS *et al.*, 2017). Likewise, H₂O₂ oxidizes phenolic compounds and alkaloids that are often found in the pericarp and seed coat and inhibit germination; therefore, this compound contributes to the breakdown of inhibitors (MERINO-VALDÉS *et al.*, 2018).

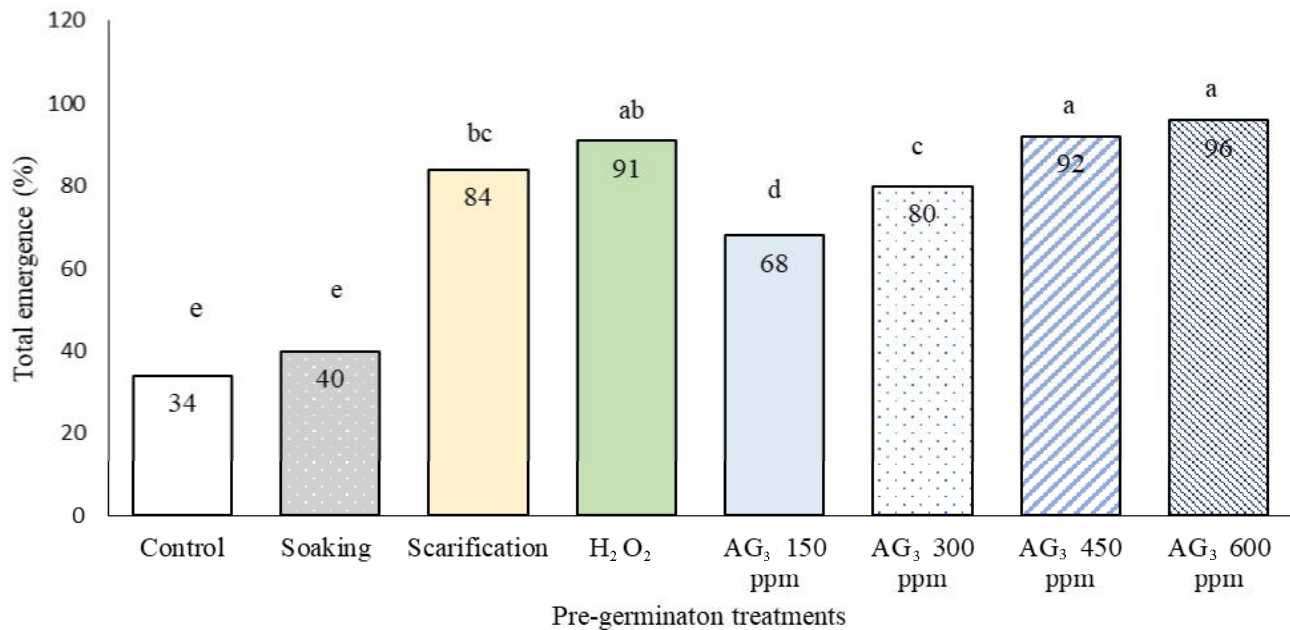
Application of AG₃ caused emergence to occur 3 to 4,5 days earlier than with the control treatment seeds (Figure 1). In some seeds, gibberellins accelerate the emergence process (CAMPOS-RUÍZ *et al.*, 2014; MERINO-VALDÉS *et al.*, 2018); however, in *E. polystachya* seeds the effect did not have notable increases. These results were similar to those obtained by Amador-Alfárez *et al.* (2013) who applied AG₃ (125, 250 and 500 mg L⁻¹) to *Ferocactus histrix* seeds and obtained germination times of 9 to 15 days. Similarly, seeds of *Solanum lycopersicum* L. variety Santa Cruz treated with 24 h of imbibition showed emergence between 11

to 15 days (DEAQUIZ-OYOLA; BURGOS-ÁVILA, 2013). The latest seedling emergence (15 days) was recorded when seeds were not treated; Viveros *et al.* (2015) report similar behavior as seedlings emerged at two weeks when *Enterolobium cyclocarpum* seeds did not receive any treatment.

In seeds of *Capsicum annum* var. *Glabriusculum*, the kinetics of imbibition were observed, finding that the seeds reached maximum imbibition at 8 h. Therefore, germination was not limited by a physical barrier to water absorption and the low germination rates were attributed to physiological seed dormancy (CANO-VÁZQUEZ *et al.*, 2015). In *E. polystachya* seeds, emergence was obtained in a similar time (8 to 15 days), excluding the scarification pretreatment, indicating water penetration into the seed was not the limiting factor; seeds soaked for 36 h had low germination (40%) due to the effect of physiological dormancy, which was overcome with the application of 150 to 600 ppm AG₃ (96% emergence), 62 and 56% more than in the control seeds and those soaked for 36 h, respectively.

Seeds treated with AG₃ at 600 ppm had the highest seedling emergence (95%), yielding 64,5% more seedlings than untreated seeds (control). Seedling emergence was higher as seeds received more AG₃. Those that produced less seedling emergence were those of the control and those that were soaked in water for 36 h (Figure 2).

Figure 2 - Total emergence of *E. polystachya* seedlings whose seeds received eight pre-germination treatments. HSD= 7,09. Means with the same letter are statistically equal according to Tukey's test ($P \leq 0,05$). HSD=Honestly significant difference



There are endogenous growth regulating hormones such as gibberellins that promote germination and induce the production of hydrolytic enzymes (MERINO-VALDÉS *et al.*, 2018). AG₃ also supplements endogenous control requirements by mobilizing seed endosperm reserves. Orantes-García *et al.* (2013) reported that pretreatment with AG₃ recorded the highest final germination percentage in three species (99% in *C. alliodora*, 93% in *B. bipinnata* and 97% in *T. amazonia*) and germination was faster. In *E. polystachya*, germination due to the effect of AG₃ was not the fastest, given that they were only in imbibition for 30 min with gibberellin, and it did not enter the seed fast enough to activate the metabolism, since, as observed in Figure 1, emergence occurred at 10 or 12 days and more soaking time is needed for imbibition to occur, similar to what happens with seeds in nature (VALLE *et al.*, 2017). Sometimes AG₃ has a negative effect on seeds, as observed by Martínez-Calderón *et al.* (2020) in *Forestiera phillyreoides* seeds, in which the use of 500 ppm caused the lowest germination value (12,5%), so sometimes seeds do not require an external stimulus to germinate.

Also with the application of H₂O₂, good total emergence (91%) was obtained in *E. polystachya*. In *F. phillyreoides* seeds, hydrogen peroxide was also used, but for a longer time (24 h) and combined with removal of the endocarp, which resulted in 95% germination (MARTÍNEZ-CALDERÓN *et al.*, 2020).

In the seeds of the present investigation, the treatment with this chemical was only for 30 min, which may have been enough time for the seeds to reach complete imbibition. Merino-Valdés *et al.* (2018) mention that hydrogen peroxide can break dormancy because it oxidizes the phenolic and alkaloid compounds found in the pericarp and seed coat, contributing to the decomposition of germination inhibitors. The O₂ also accelerates mitochondrial respiration and metabolic activities of the seed (CANO-VÁZQUEZ *et al.*, 2015).

Mechanical scarification of the seeds led to a total emergence of 84%, a treatment that allowed imbibition more easily. Similarly, Viveros *et al.* (2015) obtained high germination (81%) by sanding *Enterolobium cyclocarpum* seeds. Orantes-García *et al.* (2013) indicate that scarification had a positive effect on germination speed and final germination percentage in *C. alliodora*, *B. bipinnata* and *T. amazonia*, also highlighting that this pretreatment is economical, simple and effective in many species.

All pre-germination treatments applied to kidneywood seeds showed a positive effect, promoting a faster onset of seedling emergence and a greater number of seedlings compared to the control. This has also been observed in seeds of piquin pepper (CANO-VÁZQUEZ *et al.*, 2015), coffee (*Coffea arabica* and *C. canephora*) (ORTIZ-TIMOTEO *et al.*, 2018), *Forestiera phillyreoides* (MARTÍNEZ-CALDERÓN *et al.*, 2020), cherry (*Talisia oliviformis*) (RAMÍREZ-VILLALOBOS

et al., 2017) and manzano pepper (*Capsicum pubescens*) (MERINO-VALDÉS *et al.*, 2018).

Seedling growth

Pre-germination treatments also had a favorable effect on seedling growth (Table 2). The height of these was greater than those of the control with any of the treatments, especially in those where AG₃ was applied at concentrations of 450 to 600 ppm, as the seedlings were 4,37 to 7,25 cm taller than those from untreated seeds. Seedlings from seeds treated with the highest concentration of AG₃ had the highest growth in all variables. Compared to those of the control, stem diameter was 1,9 mm thicker, roots were 5 cm longer, the number of leaves doubled, and seedling dry matter was 68 mg higher (Table 2).

AG₃ produces activation of protein synthesis that promotes the mobilization of reserve substances (ORANTES-GARCÍA *et al.*, 2013). This promotes plant growth by cell division and elongation. This results in the growth of the hypocotyl, expansion of the cotyledons, elongation of the radicle, leaves, and inflorescences, and axial elongation of the stem, due to the increase in soluble carbohydrates available for various metabolic processes (AMADOR-ALFÉREZ *et al.*, 2013; LIMA *et al.*, 2020).

Mechanical scarification generated seedlings with good characteristics compared to those of the control; height was 4 cm greater, stem 0,9 mm thicker, roots 3 cm longer, two more leaves per seedling and 47 mg more dry matter (Table 2). Although in this treatment there was no application of gibberellins, the growth was due to the fact that these seedlings emerged first. Similarly, Viveros *et al.* (2015) obtained the greatest height (16,33 cm) and stem diameter (33 mm) of *Enterolobium cyclocarpum* (Jacq.) seedlings with mechanical seed scarification. These authors

indicate that the differences in variables such as height, stem diameter, root length, dry biomass and number of leaves are a consequence of germination speed, since plants from seeds that germinate faster tend to be larger; however, with the passage of time, growth becomes more homogeneous and the differences may disappear. Lima *et al.* (2020) state that water imbibition is a fundamental process for growth, so AG₃ at 600 ppm and scarification helped nutrient absorption, favored hormonal balance and consequently enhanced root development. In this regard, Álvarez *et al.* (2009) observed a favorable effect of gibberellins on root growth in *Chrysophyllum cainito* L. seedlings, although they used a high concentration (2000 ppm) for 24 h and had 54 cm roots.

Plants with a diameter greater than 5 mm are more resistant to bending and better tolerate drought and damage caused by predators (MARTÍNEZ-CALDERÓN *et al.*, 2020). Logically, seedling diameter will vary depending on the species; in this research with *E. polystachya*, all seedlings from the seven pre-germination treatments had a diameter greater than 2 mm and it was larger in those from seeds treated with 600 ppm AG₃, which were 1,9 mm larger than those from seeds without any treatment (Table 2).

Seedling dry matter was greater when seeds were treated with 600 ppm AG₃ (375 mg). When seed scarification was done (354 mg), there was 68 and 47 mg more dry matter than in the control seedlings, respectively (Table 2). In this regard, Barrientos *et al.* (2015); Unigarro *et al.* (2021) indicate that assimilates (carbohydrates, proteins, lipids and carbohydrates) produced by photosynthesis in the organs (mainly the leaves) can be stored or distributed via phloem among the different organs of a plant. Therefore, to increase dry matter it is important to substantially increase leaf area and achieve rapid initial growth of young plants. This explains why

Table 2 - Comparison of means for seedling growth due to the effect of pre-germination treatments on *E. polystachya* seeds at 150 days after sowing

Treatment	Height (cm)	Stem diameter (mm)	Root length (cm)	Leaves (No.)	Dry matter (g)
Control	12,12 f	2,0 f	14,87 c	10,58 e	0,307 d
Soaking for 36 h	12,81 e	2,3 d	15,13 c	12,41 d	0,335 c
Scarification	16,14 b	2,9 b	18,03 b	12,51 d	0,354 b
H ₂ O ₂	13,89 d	2,2 e	15,08 c	12,84 d	0,315 d
AG ₃ 150 ppm	13,93 d	2,1 ef	14,86 c	13,14 d	0,307 d
AG ₃ 300 ppm	14,77 c	2,4 d	14,98 c	14,83 c	0,300 d
AG ₃ 450 ppm	16,49 b	2,7 c	14,91 c	17,59 b	0,300 d
AG ₃ 600 ppm	19,37 a	3,9 a	20,07 a	21,16 a	0,375 a
HSD	0,6204	0,084	0,77	1,09	0,016

Means with the same letters in each column are statistically equal according to Tukey's test ($P \leq 0,05$). HSD=Honestly significant difference

AG₃ and scarification showed the highest dry matter accumulation in *E. polystachya* seedlings.

To successfully propagate any species of interest, it is necessary to take into account several aspects such as germination efficiency (percentage and speed), plant quality and the cost associated with each treatment (ORANTES-GARCÍA *et al.*, 2013). Therefore, for this research, the second best pretreatment that showed good seedling characteristics was mechanical seed scarification, the use of which represents a lower cost. Viveros *et al.* (2015) also conclude that seed sanding was the best treatment and recommends it to nursery operators as an alternative for the establishment of plantations or reforestation programs.

The results of this research present alternatives to break seed dormancy in *E. polystachya*: physical dormancy with scarification, and physiological dormancy with the application of AG₃ and H₂O₂. With such treatments, it will be possible to obtain a greater number of seedlings for cultivation purposes and to have plants to establish in the backyards of rural populations and in places where it grows naturally and where the habitat has been disturbed. They also contribute to the knowledge of the basic elements required to develop efficient strategies for the propagation of this species. This generates an option for plant recovery in deteriorated surfaces, with an adequate management strategy that allows the survival of plants in natural conditions. It is important to point out that it will also be possible to obtain and establish plants that in the future can be used to obtain products (leaves and stems) that will be used to cure diseases, without having to take them from plants that are in their already disturbed habitat.

The results clearly show the possibility of successfully producing kidneywood seedlings. Those obtained from this research were easily transplanted with 100% success and one year after planting started flowering. Núñez-Cruz *et al.*, 2018; Martínez-Calderón *et al.* (2020) describe kidneywood as an intermediate succession element that has considerable potential for the restoration of degraded areas in the central region of Mexico, especially where there is little water, so its use is recommended in the medium and long term.

CONCLUSIONS

1. The pretreatment that generated the best response in the germination and growth of *E. polystachya* seedlings was the application of AG₃ at 600 ppm. However, due to lower production costs, scarification is also a good alternative. The results of this research can be useful for decision making when starting production of kidneywood under greenhouse conditions or in reforestation programs, as well as for future research;

2. In five months of cultivation in the greenhouse, *E. polystachya* obtained the necessary quality parameters for use in reforestation, with a height, stem diameter, root length and number of leaves suitable for establishment in the field.

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