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Propagation of *Cunila galioides* Benth.: a Medicinal and Aromatic Species Native to South Brazil

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HIGHLIGHTS

- Methods to carry out the vegetative and sexual propagation of *C. galioides* were investigated.
- Cuttings can be used in vegetative propagation without the use of growth regulators.
- Gibberellic acid and potassium nitrate increased the germination percentage of the seeds.
- Seeds treated with gibberellic acid germinated earlier than the other treatments.

Abstract: *Cunila galioides* Benth. is an aromatic and medicinal plant widely used in the traditional medicine of South Brazil. The essential oil of one of its chemotypes has high citral contents, an oxygenated monoterpene used in perfumery, cosmetics, and food industries. The seeds of *C. galioides* have low germination percentages and there are few studies regarding its vegetative propagation. Thus, this study aimed to evaluate distinct indole-3-butyric acid concentrations (0 – control, 250, 500, and 1,000 mg·L^{-1}) on the rooting of herbaceous cuttings of *C. galioides* and different methods (gibberellic acid, potassium nitrate, and stratification) to overcome a possible dormancy in the seeds. In vegetative propagation, the use of indole-3-butyric acid 250 mg·L^{-1} only increased root score relative to the control, and higher IBA concentrations had no effective response on the cuttings, not being necessary to use IBA in *C. galioides* cuttings. The most effective treatments regarding seed germination were gibberellic acid and potassium nitrate, with germination percentages of 57.8 % and 54.5 %, respectively; stratification was not effective in increasing the germination percentage of the seeds relative to the control.

Keywords: cuttings; essential oil; germination; gibberellic acid; indole-3-butyric acid; potassium nitrate.

INTRODUCTION

The species *Cunila galioides* Benth., commonly known as 'poejo' in South Brazil, is found naturally in humid environments of the northeast upper slope of the states of Rio Grande do Sul, Santa Catarina, and Paraná [1-2]. Species of this genus are used in traditional medicine due to their stimulating, aromatic, antispasmodic, emmenagogue, antitussive, and antipyretic properties [3-4].

According to Pauletti and coauthors [4] and Fracaro and coauthors [5], *C. galioides* has three main essential oil chemotypes in which one of them has a high citral content (> 70 %). Citral, the mixture of neral and geranial isomers, is widely used in the food industry due to its citric aroma, as well as in perfumery and cosmetic industries. This compound is also used in the synthesis of β-ionone, an intermediary in the production of vitamin A [6-8]. The propagation of *C. galioides* may be carried out through sexual or asexual forms. In its native region its propagation is mainly sexual, and this species flowers between March and April [9].

Nadeem [10] and Silva and coauthors [11] commented that the study of different propagation methods is the first strategy to safeguard native medicinal species, which, due to the interest, are collected indiscriminately, increasing the risks of extinction and the heterogeneity inherent to different populations and genetic variability.

In species that produce essential oils, especially native species, due to the high variability of different chemotypes, ecotypes, and populations, vegetative propagation has great importance. Moreover, associated with clone selection, offers a quick genetic gain in genetic enhancement programs [12-13].

Cutting is a vegetative propagation method commonly used to produce seedlings of several species, being one of the most economic techniques for large-scale propagation [14-15]. The success of cutting rooting depends on the interaction of internal factors that are inherent to the species (phytohormones/plant growth regulators, reserves, hormonal balance between inhibitors, promoters, and cofactors) and external factors (environmental conditions) [15-16].

Some species root without the need of being treated with plant growth regulators, while others benefit from the exogenous application of these substances, enhancing root production. However, this benefit depends on the species and variety, cutting kind and status, and epoch of the year, among other factors [17-18]. According to Fachinello and coauthors [19], it is necessary to carry out tests with each plant species to verify the need to use plant growth regulators to induce rooting and, if necessary, the most suitable concentrations. The group of synthetic plant growth regulators most used to induce rooting is auxins, mainly indole-3-butyric acid (IBA) [20].

Sexual propagation has advantages regarding the domestication and genetic enhancement of plant species since it favors the generation of novel gene arrangements through the formation of hybrids and segregants, increasing the available genetic variability [10,21].

Although *C. galioides* produces large amounts of seeds, previous studies reported low seed viability and germinative power for this species, or the existence of dormancy [9]. Seed dormancy is described as the failure of an intact seed to germinate under favorable conditions [22-23]. For several plant species, seed dormancy is an adaptive survival strategy in hostile environments, being controlled by a large number of genes and environmental factors [24]. Commercially, seed dormancy is an undesirable trait, however, in some cases, the presence of dormancy may be advantageous, preventing earlier germination [25].

To overcome these issues, some works were conducted with several plant species, testing different methods to overcome seed dormancy. These methods aim to assess seed quality, for commercial objectives, to establish crops, or for germplasm conservation [26-27]. In this sense, several authors pointed out the role of gibberellins in seed germination, which act both in dormancy overcome and in increasing the germination speed of non-dormant seeds. These phytohormones also act in the regulation of hydrolytic enzymes, inducing the production of α-amylase, responsible for starch hydrolysis, on which the embryo depends during development [28-30]. Gibberellic acid (GA_3), an endogenous enzyme activator, and the application of this phytohormone influence protein metabolism by increasing the rate of protein synthesis in the seeds, also acting on cell elongation in most species [31].

Potassium nitrate (KNO_3) is a substance that promotes seed germination of several species; however, its mode of action is still unclear. It is believed that this substance, when in contact with components of the pericarp, potentiates gas exchanges, fostering seed germination [25,32-34].

Several species may have seed dormancy, being necessary to expose the seeds to a critical temperature, generally low (< 10 °C) before being capable to germinate; the exposition to this critical temperature triggers the occurrence of physiological and metabolic changes in the seeds. This critical temperature is not related to the optimum germination temperature and this kind of dormancy is common in species of temperate climate, with wide thermal amplitude between the cold and hot seasons [35-37].

Works have shown that dormancy overcoming, regardless of the dormancy mechanism, is related to a quick decrease in the content of abscisic acid (ABA) in seed tissues. This phytohormone appears to be synthesized during seed dormancy, and its degradation occurs when dormancy is overcome [24].

Thus, this work aimed to evaluate the germinative potential of *Cunila galioides* Benth. seeds using treatments to overcome a possible seed dormancy, as well as to raise subsidies to carry out the vegetative propagation of this species evaluating the effect of indole-3-butyric acid (IBA) on cuttings.

MATERIAL AND METHODS

The experiments were conducted in the Laboratory of Seeds of the Department of Forage Plants and Agrometeorology and the greenhouse of the Department of Horticulture and Silviculture of the Faculty of Agronomy of the Federal University of Rio Grande do Sul. The cuttings were collected in March 2003, and the seeds in May 2004, from plants of the 'André da Rocha' population, with citral chemotype [4]. This plant population was chosen because it had the best performance regarding essential oil production in a previous study [4]. Vouchers were deposited in the Bank of Germplasm of Aromatic and Medicinal Species of the Laboratory of Essential Oils, in the Institute of Biotechnology of the University of Caxias do Sul.

The first experiment evaluated the effect of increasing doses of IBA in the rooting of *C. galioides* cuttings. Apical cuttings of young plant matrices, with a length of 10 cm and the bottom third defoliated, had the lowest 2 cm immersed in an aqueous solution of IBA for 1 h. The IBA solution was prepared by dissolving IBA in 30 mL ethanol and distilled water was added up to 1 L.

The treatments tested were zero (control), 250, 500, and 1,000 mg·L^{-1} IBA. Carbonized rice husk was used as the substrate, being put in plastic trays with 55.0 cm x 35.5 cm x 10.0 cm with holes in the inferior part for water drainage. The cuttings were kept under intermittent irrigation by nebulization (2 min of nebulization every 30 min) for 45 days, being evaluated after this period.

The following parameters were evaluated: number of roots per cutting, aerial increase (%), dry mass of aerial and root parts (mg), and qualitative evaluation of root development through a score scale. The score was determined as 1 = small root with up to 15 rootlets in each cutting; 3 = average root development with 16 to 25 rootlets in each cutting; 5 = longer and well-developed root system, with more than 25 rootlets per cutting. The aerial increase was calculated using equation 1.

$$\text{AI (\%)} = \frac{100 \times \text{FL}}{\text{SL}} \tag{1}$$

Being 'AI' the aerial increase, 'FL' the length of the aerial part of the cuttings after 45 days (cm), and 'SL' the length of the aerial part of the cuttings at the start of the experiment (cm).

The experiment was conducted following a completely randomized design with four treatments (IBA concentrations) and 12 cuttings per parcel with four replicates, totaling 48 cuttings per treatment.

In the second experiment, which evaluated the sexual propagation of *C. galioides*, after the harvest of the seeds, they were dried in a sieve with filter paper, at room temperature (20-25 °C) for 15 days. A germination chamber was used to carry out the test at a constant temperature of 20±3 °C and a photoperiod of 16 h of light and 8 h of dark. The seeds were put to germinate in crystal plastic boxes with 11.5 x 11.5 x 3.5 cm, on two sheets of blotting paper [34].

The experimental design was completely randomized, with 100 seeds per replicate with four replicates, totaling 400 seeds per treatment. Four treatments were tested: T1 – control; T2 – application of gibberellic acid (GA_3) 250 mg·L^{-1}; T3 – application of potassium nitrate (KNO_3) 0.1% w/v; T4 – cold stratification (5 °C for 7 days). These treatments were based on the Rules for Seed Analysis [34]. In treatments T3 and T4, the application of GA_3 or KNO_3 was carried out in the first irrigation of the seeds. In treatments T1 (control) and T4 (cold stratification), the first irrigation was carried out using distilled water. The starting irrigation volume was 6.5 mL, which corresponded to three times the weight of blotting paper [38]. To keep humidity, the boxes were daily irrigated with distilled water, as required.

The germination experiment was conducted for 21 days, and the parameters of germination percentage, germination speed index (GSI), and evolution of germination over time were evaluated. Germination percentage was determined using equation 2.

$$\text{G (\%)} = 100 \times \frac{n_i}{N} \tag{2}$$

Being 'n_i' the number of seeds that germinated on day 'i' and 'N' the number of seeds in the replicate. GSI values were determined using equation 3, proposed by Maguire [39].

$$GSI = \sum_{i=1}^{21} \frac{G_i}{N_i} \quad (3)$$

Being ‘G_i’ the number of seeds that germinated on the day ‘i’, and ‘N_i’ the number of days after sowing, evaluated every 7 days up to 21 days.

The results of both experiments underwent Levene’s test (homoscedasticity) and Shapiro-Wilk’s test (homogeneity of residuals), followed by Analysis of Variance (ANOVA) and Tukey’s multiple range test at 5 % probability (α = 0.05). Germination percentage data were transformed using the arcsine of the square root of x/100.

RESULTS AND DISCUSSION

Effect of increasing concentrations of indole-3-butyric acid (IBA) on the rooting and development of *C. galioides* plants

The percentage of rooted cuttings was quite high, above 95 %, in all treatments, showing that the *C. galioides* population used in the study roots easily. The cuttings were collected from the apical region of young herbaceous plant matrices in a full vegetative state, which, according to Trobec and coauthors [16] have a better rooting capacity. It is also important to consider that the presence of young leaves in the cutting is responsible for the production of auxins that are naturally found in the plant, thus increasing the availability of endogenous auxins for rooting and plant development [19].

The results of the production parameters of the cuttings, evaluated 45 days after the start of the experiment, are presented in Table 1.

Table 1. Results of the evaluated production parameters of *C. galioides* plants treated with increasing indole-3-butyric acid (IBA) concentrations.

IBA concentration ($mg \cdot L^{-1}$)	Number of roots	Root score	Dry root mass (mg)	Dry aerial mass (mg)	Aerial increase (%)
zero (control)	19 a	2.22 b	82 a	440 a	122 a
250	22 a	2.95 a	75 a	420 a	118 a
500	21 a	2.23 b	80 a	390 ab	90 ab
1,000	17 a	1.88 b	50 a	290 b	40 b
Coefficient of variation (%)	11.6	18.5	22.0	20.1	17.7

Means followed by the same letter do not differ statistically by Tukey’s multiple range test at 5 % probability (α = 0.05).

It was possible to observe distinct responses relative to each parameter evaluated and the IBA concentration applied to the cuttings. Regarding the number of roots and dry root mass, there were no statistical differences between the treatments. However, an important parameter is the root score, which gives qualitative information about root characteristics that are associated with its development [40]. Regarding this parameter, the cuttings treated with IBA 250 $mg \cdot L^{-1}$ had higher root scores (Figure 1) than the control and the other IBA concentrations, which have not differed between themselves. For aerial increment, the control and 250 $mg \cdot L^{-1}$ IBA were statistically superior to 1,000 $mg \cdot L^{-1}$ IBA, whereas the treatment with 500 $mg \cdot L^{-1}$ IBA had an intermediate performance.

A B

Figure 1. Rooting experiment of *C. galioides cuttings* (A) and the root of the treatment IBA 250 $mg \cdot L^{-1}$ (B), which had the best root score among all treatments.

Pauletti and coauthors [4], in a previous work, treated *C. galioides* cuttings with IBA 250 mg·L^{-1}, aiming to propagate plants from different populations to determine essential oil composition and yield. Loconsole and coauthors [45], tested the effect of increasing IBA concentrations (0 – 5,000 mg·L^{-1}) on the rooting of *Lantana camara* L. and *Abelia x grandiflora* (Andrè) Rehd. Cuttings. They observed that concentrations greater than 1250 mg·L^{-1} had a negative effect root development of *A. grandifolia*, but the cuttings treated with the concentration of 1250 mg·L^{-1} IBA (the lowest concentration tested) had the better root parameters (root length, number of rootlets) than other treatments. For *L. camara*, the root response was linear and positively related with IBA concentration, with better root parameters at the highest IBA concentration (5,000 mg·L^{-1}). This is a strong indicative of the individual susceptibility of each species regarding the use of plant growth regulators, even for rooting purposes.

The relationship between the aerial dry plant mass of *C. galioides* cuttings and the increasing concentrations of IBA is presented in Figure 2.

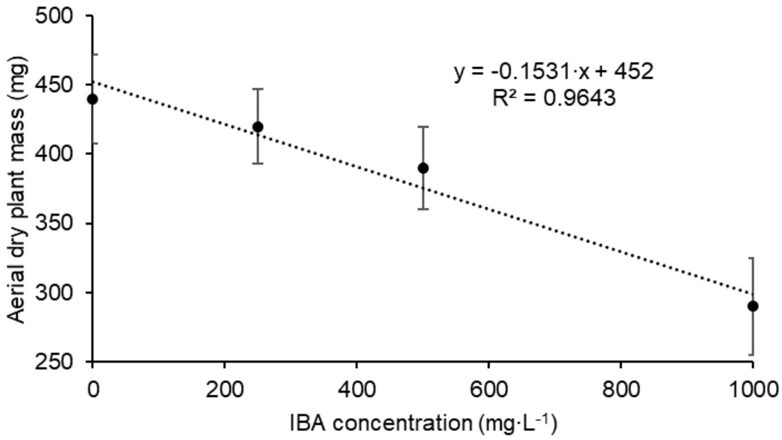

Figure 2. Dry mass of the aerial part of *C. galioides* cuttings treated with increasing doses of indole-3-butyric acid (IBA) 45 days after cutting.

It was observed a linear decrease in the production of the aerial dry mass as the IBA concentration used in the cuttings increased; this decrease was more accentuated from the concentration of 500 mg·L^{-1}. This fact may indicate a phytotoxic effect of IBA, probably due to the longer exposure time of the cuttings to the auxin (1 h), which may be too long when higher IBA concentrations are used [19]. However, Balestrin and coauthors [46], testing increasing IBA concentrations on the rooting of *Rubus erythroclados* Mart. ex Hook. f. cuttings, observed that IBA concentrations up to 5,000 mg·L^{-1} had no significant effect on the production of dry aerial mass, nor on root length.

Although not evident, the differences between the treatments for the number of roots and dry root mass, and the differences regarding the aerial increase between the control and IBA 250 mg·L^{-1} may suggest that using this growth plant regulation at this concentration helps to enhance the rooting of *C. galioides* cuttings because of the better root score (Table 1), which is an important qualitative parameter. However, the observed results indicate that the vegetative propagation of *C. galioides* may be carried out through cuttings without the need for using exogenous auxins, which is important and desirable from technical and economic standpoints in large-scale production of seedlings.

Effect of different dormancy overcoming methods on the germination of *C. galioides* seeds

Regarding seed germination, the observed results showed that the use of the plant growth regulator from the group of gibberellins (GA_3), as well as KNO_3, increased the germination percentages (Figure 3A) and germination speed (Figure 3B) of *C. galioides* seeds. Cold stratification at 5 °C for 7 days was not efficient in influencing neither the germination percentage nor the germination speed, with performance statistically similar to the control in both parameters. The ineffectiveness of this treatment may be related to a lower moisture content of the seeds during the stratification. According to Zaidan and Barbedo [41], the method of dormancy overcoming through low temperatures required the arrangement of the seeds between layers of a moistened substrate.

The results of average germination percentage after 21 days and germination speed index for *C. galioides* seeds treated with different dormancy overcoming methods are presented in Figure 2.

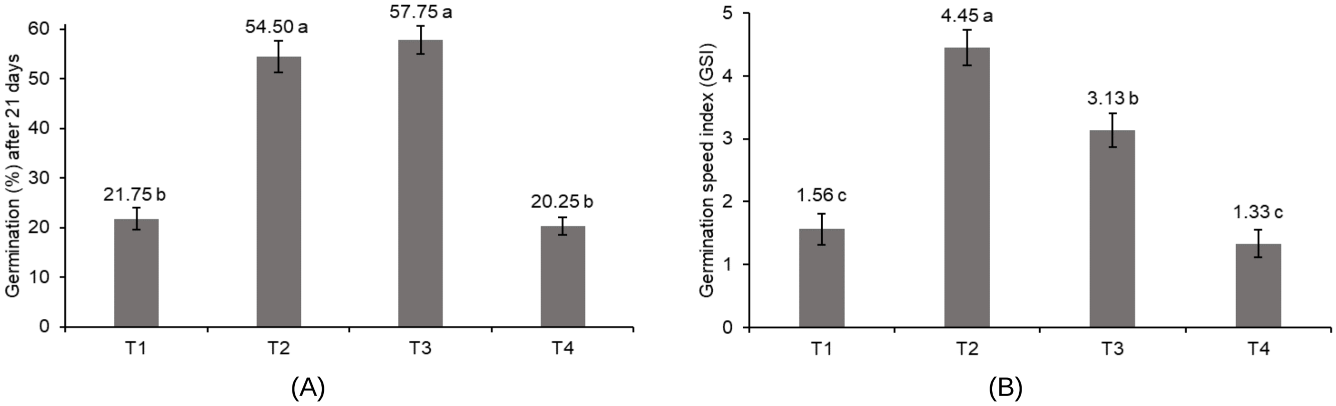

Figure 3. Average germination percentage after 21 days (A) and germination speed index (GSI, B) of *C. galioides* seeds treated with different dormancy overcoming methods. Means followed by the same letter do not differ statistically by Tukey's multiple range test at 5 % probability (α = 0.05). T1 – control; T2 – gibberellic acid 250 mg·L^{-1}; T3 – KNO3 0.1 % w/v; T4 – cold stratification (5 °C for 7 days). (A – CV = 30 %; B – CV = 52 %).

Ayoyama and coauthors [35] observed similar behavior in *Lavandula angustifolia* Miller seeds, an aromatic species from the Lamiaceae family, in which the application of GA_3 (100 and 200 mg·L^{-1}) was efficient in increasing germination percentages and cold stratification (7 °C for 7 days) have not differed from the control (20.3 % and 21.8 %, respectively).

Grigoriadou and coauthors [42], evaluating propagation protocols for the replication and conservation of 22 different species of aromatic and medicinal plants from the Mediterranean region, reported that the use of auxins helped several species in increasing the germination efficiency of seeds and the rooting of cuttings. However, the same authors also stated that the need for a specific plant growth regulator, the optimum concentration, and exposure time is highly dependent on the species, and also on the variety due to intraspecies genetic variability.

Elhindi and coauthors [43], investigating the effect of the plant growth regulators gibberellic acid (GA_3), indole-3-acetic acid (IAA), indol-3-butyric acid (IBA), and naphthalene acetic acid (NAA) on the seed germination of *Mentha piperita*, *Ocimum basilicum*, and *Coriandrum sativum*, reported that GA_3 was the most effective regulator regarding seed germination of the tested species. However, there was wide variability between species regarding seed sensitivity to the growth regulators and the germinative potential of the untreated seeds.

Literature has reports of works showing the gibberellins and some chemicals, such as KNO_3, as effective to overcome seed dormancy or to increase the germination percentage and speed in some species [25,44]. This was observed in this work, in which the treatments that induced the highest germination percentages were GA_3 and KNO_3, and regarding the germination speed, GA_3 had the higher GSI (4.45), followed by KNO_3 (3.13).

The evolution of the germination percentages of *C. galioides* seeds in the 21 days of the germination test is shown in Figure 4.

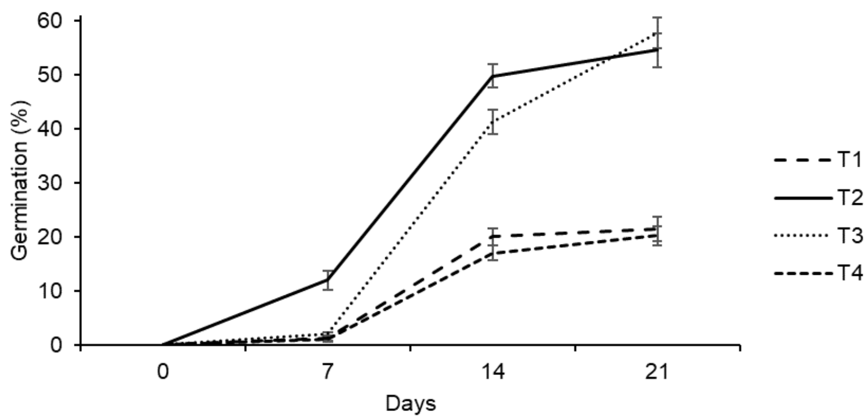

Figure 4. Accumulated germination percentages of *C. galioides* seeds after treatment with different dormancy overcoming methods. T1 – control; T2 – gibberellic acid 250 mg·L^{-1}; T3 – KNO3 0.1 % w/v; T4 – cold stratification (5 °C for 7 days).

In the first two evaluations, on the days 7 and 14, the use of GA_3 250 mg·L^{-1} induced higher germination percentages relative to the other treatments, which can also be seen by the higher GSI values (Figure 3B) regarding this treatment. This suggests that the exposition of the seeds to GA_3 may have increased the seedling vigor. In this sense, several authors commented that the application of gibberellins increases germination percentage and speed by enhancing tissue metabolism, increasing the seed vigor [30-31,43]. Images of the seed germination test after 21 days are presented in Figure 5.

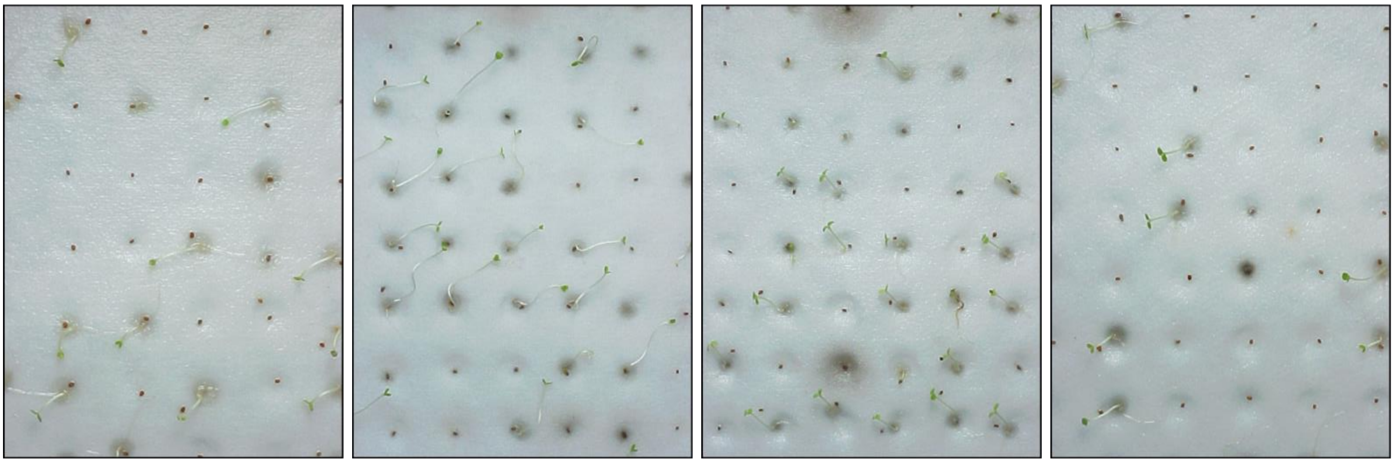

Figure 5. Germination of *C. galioides* seeds 21 days after treatment with different dormancy overcoming methods. T1 – control; T2 – gibberellic acid (GA_3) 250 mg·L^{-1}; T3 – KNO_3 0.1 % w/v; T4 – cold stratification (5 °C for 7 days).

However, Tafarel and coauthors [22-23], who studied the use of different dormancy overcoming methods in the seeds of *Eugenia pyriformis* Cambess and *Psidium cattleianum* Sabine, reported that the use of gibberellic acid 500 mg·L^{-1} did not affect germination percentage nor germination speed of the treated seeds relative to the control. This highlights the individual sensitivity of each species to a specific plant growth regulator or dormancy overcoming method.

CONCLUSION

According to the results, herbaceous cuttings of *C. galioides*, with a length of 10 cm, may be used in the vegetative propagation of this species, without the use of indole-3-butyric acid (IBA), albeit using it at the concentration of 250 mg·L^{-1} may help enhance cutting rooting. The germination of *C. galioides* seeds may be enhanced by using gibberellic acid 250 mg·L^{-1} or potassium nitrate 0.1 % w/v as effective dormancy overcoming methods. For faster seed germination, the use of gibberellic acid 250 mg·L^{-1} may reduce the germination time by approximately 7 to 14 days relative to untreated seeds.

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Conflicts of Interest: The authors declare no conflict of interest.

REFERENCES

1. Bordignon SAL. [Botanical and chemical study of *Cunila royen* ex L. (Lamiaceace) species native to Southern Brazil] [thesis]. Porto Alegre (Brazil): Universidade Federal do Rio Grande do Sul; 1997. Portuguese.
2. Pauletti GF, Barros IBI, Echeverrigaray S, Atti-Serafini L, Rota LD, Santos ACA. [Quantitative analysis of the production of essential oils of *Cunila galioides* Benth. (poejo) plants grown in Rio Grande do Sul]. Proceedings of the 43th Congresso Brasileiro de Olericultura; 2003; Recife, Brazil. Recife: Associação Brasileira de Olericultura; 2003. Portuguese.
3. Simões CMO, Mentz LA, Schenkel EP, Irgang BE, Stehmann JR. [Plants of traditional medicine of Rio Grande do Sul]. 5th ed. Porto Alegre: Editora da UFRGS; 1994. Portuguese.
4. Pauletti GF, Silvestre WP, Rota LD, Echeverrigaray S, Barros IBI. Poejo (*Cunila galioides* Benth.) production in Five Agroecological Regions of Rio Grande do Sul. Braz Arch Biol Technol. 2020;63:e20190481.
5. Fracaro F, Serafini LA, Santos ACA, Paroul N, Echeverrigaray S. Analysis of the essential oil composition of *Cunila galioides* Benth. J Essent Oil Res. 2002;14:336-8.
6. Bauer K, Garbe D, Surburb H. Common Fragrance and Flavor Materials: Preparation, Properties and Uses. Berlin: Wiley-VCH; 2001.

7. Sadraei H, Ghannadi A, Malekshhi K. Relaxant effect of essential oil of *Melissa officinalis* and citral on rat ileum contractions. Fitoterapia. 2003;74:445-52.
8. Sharma S, Habib S, Sahu S, Gupta J. Chemical Properties and Therapeutic Potential of Citral, a Monoterpene Isolated from Lemongrass. Med Chem. 2021;17(1):2-12.
9. Fracaro F, Echeverrigaray S. Micropropagation of *Cunila galioides*, a popular medicinal plant of south Brazil. Plant Cell Tissue Organ Cult. 2001;64:1-4.
10. Nadeem M, Palni LMS, Purohit AN, Pandey H, Nandi SK. Propagation and Conservation of *Podophyllum hexandrum* Royle: an important medicinal herb. Biol Conserv. 2000;92:121-9.
11. Silva RC, Belniaki AC, Vieira ESN, Cuquel FL, Panobianco M. Subsidies for propagation of native species in Brazil with medicinal potential: *Calophyllum brasiliense* Cambess. J Seed Sci. 2019;41(3):318-27.
12. Mesén F, Newton AC, Leakey RRB. Vegetative propagation of *Cordia alliodora* (Ruiz & Pavon) Oken: the effects of IBA concentration, propagation medium and cutting origin. For Ecol Manag. 1997;92:45-54.
13. Kettenhuber PW, Sousa R, Sutili F. Vegetative Propagation of Brazilian Native Species for Restoration of Degraded Areas. Floresta e Ambiente, v. 26, n. 2, e20170956, 2019.
14. Fernandes AA, Martinez HEP, Silva DJH, Barbosa JG. [Production of tomato seedlings through cuttings rooted in hydroponics]. Pesq Agropec Bras. 2004;39(4):343-8. Portuguese.
15. Wu HX. Benefits and risks of using clones in forestry – a review. Scand J For Res. 2019;34(5):352-9.
16. Trobec M, Satampar F, Veberic R, Osterc G. Fluctuations of different endogenous phenolic compounds and cinnamic acid in the first days of the rooting process of cherry rootstock ‘GiSelA 5’ cuttings. J Plant Physiol. 2005;162(5):589-97.
17. Hartmann HT, Kester DE, Davies FT. Plant propagation: principles and practices. 5th ed. New Jersey: Pentice-Hall International; 1990.
18. Motte H, Vanneste S, Beeckman T. Molecular and Environmental Regulation of Root Development. Annu Rev Plant Biol. 2019;70:465-88.
19. Fachinello JC, Kersten E, Hoffmann A, Nachitigal JC, Fortes GRL. [Propagation of temperate fruit trees]. Pelotas: UFPEL; 1995. Portuguese.
20. Khandaker MM, Saidi A, Badaluddin NA, Yusoff N, Majrashi A, Alenazi MM, et al. Effects of Indole-3-Butyric Acid (IBA) and rooting media on rooting and survival of air layered wax apple (*Syzygium samarangense*) CV Jambu Madu. Braz J Biol. 2022;87:e256277.
21. Rossum FV, Hardy OJ. Guidelines for genetic monitoring of translocated plant populations. Conserv Biol. 2022;36:e13670.
22. Tafarel AZ, Silvestre WP, Pansera MR, Rodrigues LS, Sartori VC. Seed dormancy and germination in *Psidium cattleyanum* Sabine (red and yellow araçá). Rev Interdiscip Cien Apl. 2021;5(9):20-7.
23. Tafarel AZ, Silvestre WP, Pansera MR, Rodrigues LS, Sartori VC. Seed dormancy and germination in Uvaia (*Eugenia pyriformis* Cambess). Rev Interdiscip Cien Apl. 2021;5(9):51-6.
24. Koornneef M, Bentsink L, Hilhorst H. Seeds dormance and germination. Curr Opin Plant Biol. 2002;5(1):33-6.
25. Gubler F, Millar AA, Jacobsen JV. Dormancy release, ABA and pre-harvest sprouting. Curr Opin Plant Biol. 2005;8(2):183-7.
26. Faron MLB, Percin MB, Lago AA, Bovi AO, Maia NB. [Temperaure, potassium nitrate, and photoperiod on the germination of *Hipericum perforatum* L. and *H. brasiliense* Choisy seeds]. Bragantia. 2004;63(2):193-9. Portuguese.
27. Beveridge FC, Williams A, Adkins SW. Seed enhancement technologies to improve germination and emergence of Australian native Poaceae. Seed Sci Res. 2020;30:293-303.
28. Ynoue CK, Ono EO, Marchi LOS. [Effect of GA_3 on the germination of kiwi (*Actinidia chinensis* Planch.) seeds]. Sci Agric. 1999;56(1):9-12. Portuguese.
29. Martins L, Silva WR. [Behavior of seed doramancy of brachiaria seed submitted to thermal and chemical treatments]. Pesq Agropec Bras. 2001;36(7):997-1003. Portuguese.
30. Stenzel NMC, Murata IM, Neves CSVJ. [Dormancy overcoming in atemoya and and sugar-apple seeds]. Rev Bras Frutic. 2003;21(2):305-8. Portuguese.
31. Aragão CA, Dantas B, Alves E, Cataneo AC, Cavariani C, Nakagawa J. [Amylolytic activity and physiological quality of stored ‘super sweet corn’ seeds treated with gibberellic acid]. Rev Bras Sem. 2003;25(1):43-8. Portuguese.
32. Leonel S, Rodrigues JD. [Effects of gibberellins, cytokinins, and potassium nitrate on the germinative process of rangpur (*Citrus x limonia* Osbeck) seeds]. Sci Agric. 1999;56(1):111-6. Portuguese.
33. Lula AA, Alvarenga AA, Almeida LP, Alves JD, Magalhães MM. [Studies of chemical agents on the overcoming of dormancy in *Paspalum paniculatum* L. seeds]. Cien Agrotecnol. 2000;24(2):358-66. Portuguese.
34. Brasil, Ministério da Agricultura e Reforma Agrária. [Rules for seed analysis]. Brasília: Departamento de Defesa Vegetal; 2009. Portuguese.
35. Ayoyama EM, Ono EO, Furlan MR. [Study of the germination of lavender (*Lavandula angustifolia* Miller) seeds]. Sci Agric. 1996;53(2-3):267-72. Portuguese.
36. Flanigan NP, Bandara R, Wang F, Jastrzezbowski S, Hidayati SN, Walck JL. Germination responses to winter warm spells and warming vary widely among woody plants in a temperate forest. Plant Biol. 2020;22:1052-61.

37. Shi L, Teixeira CSP, Dance T, Sullivan JA, Lyons EM. The effect of temperature and cold-moist stratification on seed germination of temperate climate grass and forb prairie species. Proceedings of the 20th Agronomy Australia Conference, Toowoomba, Australia; 2022.
38. Alves MCS, Medeiros Filho S, Innecco R, Torres SB. [Allelopathy of volatile extracts on seed germination and root length of lettuce]. Pesq Agropec Bras. 2004;39(11):1083-6. Portuguese.
39. Maguire JD. Speed of germination aid in selection and evaluation for seedling emergence and vigor. Crop Sci. 1962;2(1):176-7.
40. Pardo VA, Barros IBI. [Vegetative propagation of marcela (*Achyrocline satureioides*) under different rooting periods and doses of índole-3-butyric acid]. Pesq Agropec Gaúcha. 1997;3(1):41-8. Portuguese.
41. Zaidan LBP, Barbedo CJ. [Dormancy overcoming in seeds]. In: Ferreira AG, Borguetti F, editors. [Germination: from basics to application]. Porto Alegre: Artmed; 2004. p. 135-146.
42. Grigoriadou K, Krigas N, Sarropoulou V, Papanastasi K, Tsoktouridis G, Maloupa E. In vitro propagation of medicinal and aromatic plants: the case of selected Greek species with conservation priority. In Vitro Cell Develop Biol Plant. 2019;55:635–46.
43. Elhindi KM, Dewir YH, Asrar AW, Abdel-Salam E, El-Din AS, Ali M. Improvement of Seed Germination in Three Medicinal Plant Species by Plant Growth Regulators. HortSci. 2016;51(7):887-91.
44. Peng J, Harberd NP. The role of GA-mediated signaling in the control of seed. Curr Opin Plant Biol. 2002;5:376-81.
45. Loconsole D, Cristiano G, De Lucia B. Image Analysis of Adventitious Root Quality in Wild Sage and Glossy Abelia Cuttings after Application of Different Indole-3-Butyric Acid Concentrations. Plants. 2022;11:290.
46. Balestrin, JT, Baroni T, Rogalski JM. Effect of Cutting Size on the Performance of Exogenous Auxin IBA in Vegetative Propagation of *Rubus erythroclados* Mart. ex Hook. f. J Exp Agr Int. 2021;43(8): 56-66.